European Union Reference Laboratory for Fish and Crustacean Diseases
NATIONAL INSTITUTE OF AQUATIC RESOURCES, TECHNICAL UNIVERSITY OF DENMARK

EURL for Fish Diseases

Report of the Inter-Laboratory Proficiency Test 2022

for identification and titration of

VHSV, IHNV, EHNV, SVCV and IPNV (PT1)

and identification of

CyHV-3 (KHV), SAV and ISAV (PT2)

Organised by the
European Union Reference Laboratory for Fish and Crustacean Diseases,
National Institute of Aquatic Resources, Technical University of Denmark,
Kgs. Lyngby, Denmark

ilac-MRA

DANAK
PT Reg.No. 515
Member of EA MLA

Table of contents

Introduction

A comparative test of diagnostic procedures was provided by the European Union Reference Laboratory (EURL) for Fish and Crustacean Diseases. The test was divided into proficiency test 1 (PT1) and proficiency test 2 (PT2).
PT1 was designed to assess the ability of participating laboratories in quantifying and identifying the fish viruses causing notifiable diseases: viral haemorrhagic septicaemia virus (VHSV), infectious hematopoietic necrosis virus (IHNV), and epizootic haematopoietic necrosis virus (EHNV) or related rana-viruses and in addition other fish pathogenic viruses as pike fry rhabdovirus (PFR), spring viraemia of carp virus (SVCV) and infectious pancreatic necrosis virus (IPNV). The laboratory procedures for isolating and titrating these pathogens is primarily based on cell culture methods, however the use of molecular methods (Real Time PCR based) has been implemented for their detection and identification.
PT2 was designed to assess the ability of participating laboratories to identify the fish viruses: infectious salmon anaemia virus (ISAV), salmonid alphavirus (SAV) and cyprinid herpesvirus 3 (CyHV-3) (otherwise known as koi herpes virus – KHV) by molecular methods (PCR based).
Out of the 42 laboratories participating in PT1, 30 performed analysis to identify all viruses included, while out of the 41 laboratories participating in PT2, 33 attempted to identify all fish viral pathogens included.
The tests were sent from the EURL 5th of October 2022.
Both PT1 and PT2 are accredited by DANAK under registration number 515 for provision of proficiency testing according to the quality assurance standard DS/EN ISO/IEC 17043.
The EURL relies on the subcontractor Eurofins Genomics for sequencing the amplicons of viral isolates included in the PTs, DTU – National Food Institute for lyophilisation of the ampoules and the Danish National Reference Laboratory for Fish diseases as provider of cell cultures.
This report covers both the results of PT1 and PT2.

PT1 consisted of five coded ampoules (I-V). These ampoules contained IHNV (high titter), ECV, SVCV, VHSV and IHNV (low titter), respectively (see table 1).

The proficiency test is designed to primarily assess the ability of participating laboratories to identify fish viral pathogens causing diseases listed in **Commission Implementing Regulation (EU) 2018/1882** [1].

PT1 include the Category A disease, EHN, for which it is necessary to distinguish by sequencing the causative agent, EHNV, from other ranavirus, and the Category C diseases VHS and IHN. Furthermore the inter-laboratory proficiency test is also suitable for maintaining accreditation for identification of SVCV, and IPNV. Finally, participants have to consider that the test ampoules also could contain other viruses (e.g. other fish rhabdoviruses, ranaviruses, or birnaviruses). The participants were also asked to quantify the viruses in PT1 by titration in order to assess the susceptibility of their fish cell lines for virus infection. Participants were encouraged to use their normal standard laboratory procedures. However, the identification should be performed according to the procedures laid down in diagnostic manuals for listed fish diseases available on the EURL website https://www.eurl-fish-crustacean.eu/fish/diagnostic-manuals and on the instruction to participants delivered along with the parcel [2] and by using fish cell cultures followed by e.g. ELISA, PCR or immunofluorescence (IFAT).

If ranavirus was present in any of the ampoules, it was mandatory to perform sequence analysis according to the manual provided on the EURL website https://www.eurl-fish-crustacean.eu/fish/diagnostic-manuals . Although sequencing is necessary, it is possible to perform a corroborative test with restriction endonuclease analysis (REA) of the isolate in order to determine if

the isolate was EHNV or another ranavirus and it was recommended to follow the procedures described in Chapter 2.3.2 in the WOAH Manual of Diagnostic Tests for Aquatic Animals [3]. Laboratories were encouraged to further characterize VHSV and IHNV isolates by means of genotyping. It was recommended to use the genotyping procedure described in Einer-Jensen 2004 [4] for VHSV and ; for IHNV, we suggest to follow procedure provided in the latest IHNV chapter of the WOAH manual on Aquatic Animal Diseases (primer references are given in Emmenegger et al. (2000) [5], and PCR conditions are given in Garver et al. (2003) [6]. Laboratories were encouraged to submit all sequencing results used for genotyping the isolates.

PT2 consisted of four coded ampoules (VI-IX). These ampoules contained BF-2, KHV, ISAV and SAV cell supernatant, respectively (see table 11). The test was designed to primarily assess the ability of participating laboratories to identify infection with HPR-deleted ISAV listed as category C disease, , and Koi herpes virus disease listed as category E diseases (Commission Implementing Regulation (EU) 2018/1882**[1]**) if present in the ampoules, bearing in mind that the test ampoules could also contain other pathogens. Since SAV is not a listed disease in the European legislation, all participants were free to decide if they would be testing for SAV or not. Each participant was asked to declare whether they would test for SAV or not. The EURL team would then take care of calculating the score accordingly, overall 36 of 41 laboratories tested for SAV in 2022.
Participants were expected to use their normal PCR or real-time PCR methods for detection of the pathogens. Regarding SAV analysis, participants can refer to the Chapter 2.3.8. of the WOAH Manual of Diagnostic Tests for Aquatic Animals [7]. It was not mandatory to grow KHV and ISAV on cell cultures in this test, but the viruses were not inactivated and, thus in theory, it should be possible to propagate them in cell cultures.

The EURL has acknowledged the big effort that many participants are putting in sequencing and genotyping the isolates of the PT panel, for this reason, the genotyping results provided by all participants is displayed in Table 10 and 15.

Finally, in the attempt to harmonize the molecular diagnostic methods the EURL has compiled and presented the Ct values reported by the different laboratories (table 9 and figure 10 for PT1; table 14 and figure 14 for PT2).

Each laboratory was given a code number to ensure discretion. The code number of each participant is supplied to the respective laboratories with this report. Furthermore, the EURL-team has included comments to the participants if relevant. An un-coded version of the report is sent to the European Commission.

Participants were asked to download an excel sheet from the EURL web site (https://www.eurl-fish-crustacean.eu/) to be used for reporting results and to be submitted to the EURL electronically. Participants were asked to reply latest December 18th 2022. The results of the inter-laboratory proficiency test for listed fish diseases 2022 and plans and idea for future inter-laboratory tests will be presented at the 27th Annual Workshop of the NRLs for Fish Diseases on May 30th and 31st 2023. Furthermore a specific online meeting in April will be organized to discuss the report and receive comments, inputs and feedback from the participating laboratory.

Distribution of the test

The test was sent out according to current international regulations for shipment of diagnostic specimens UN 3373, "Biological substance, Category B". All proficiency test parcels were delivered by courier. When possible participants were provided with a tracking number so they were able to follow the shipment.

Shipment and handling
The parcels were delivered to 29 participants within the first week; 90% were delivered within the first two weeks (Figure 1). All the parcels were sent without cooling elements.
A relatively high stability was demonstrated to characterize the lyophilized pathogens in glass ampoules as described in proficiency test reports 2007,2010,2011.

Extra parcels were kept at approx. 4°C in order to be able to provide fast substitutes in case of damage during transport. Do to transport challenges, it was necessary to provide a new package for two countries, one participant didn't receive the parcel due to internal clearance issues.

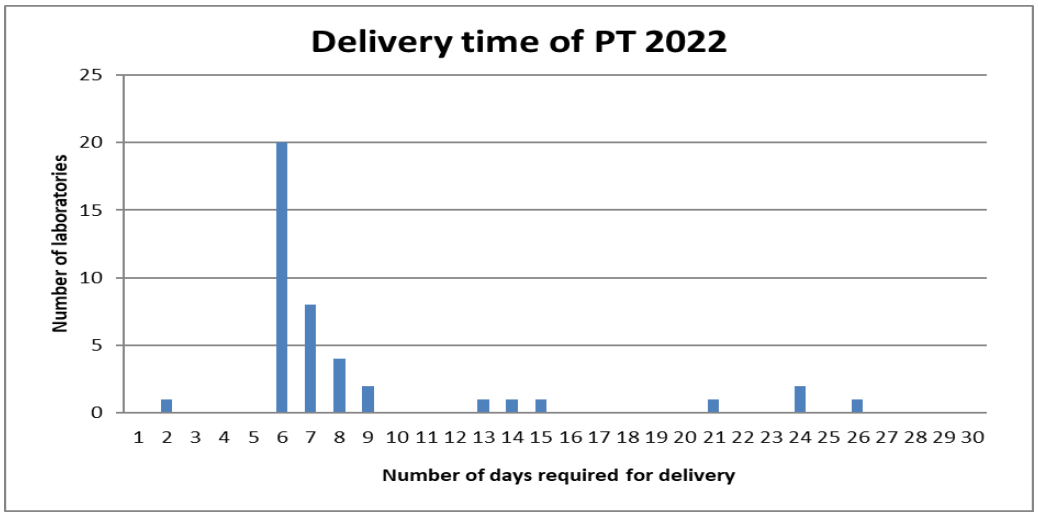


Figure 1. Transport time for the parcels to reach the participants.

Participation

PT1 and PT2: 42 laboratories received the annual proficiency test. 42 participants submitted the full spreadsheet within the deadline. Figure 2 show the numbers of participants in the proficiency test from 2010 to 2022.

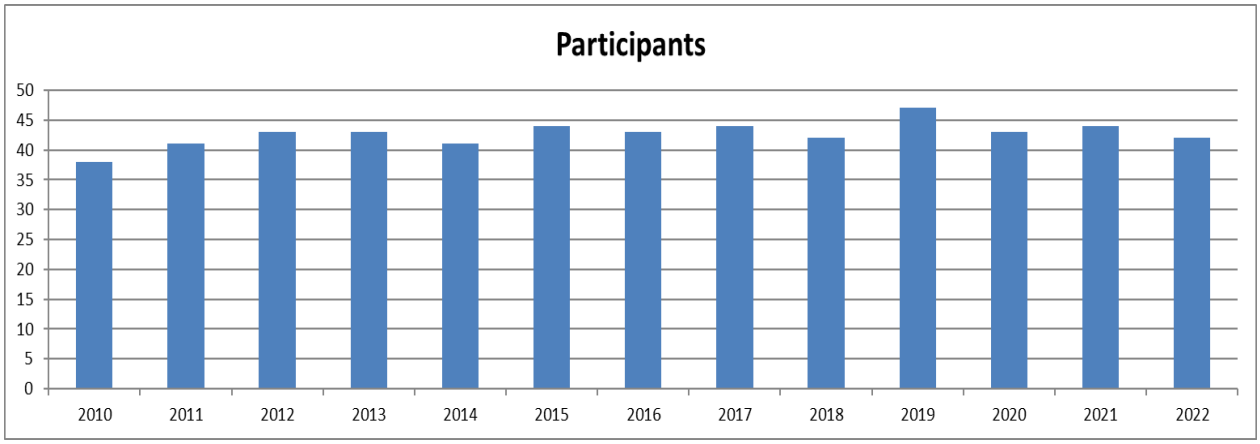


Figure 2. Participants in the EURL proficiency test over the years.

Proficiency test 1, PT1

Five ampoules with lyophilised cell culture supernatant were delivered to all NRLs in the EU Member States including Denmark and likewise to the NRLs in Australia, Bosnia and Herzegovina, Canada, Faroe Islands, Iceland, Japan, New Zealand, Northern Ireland, Norway, Republic of North Macedonia, Serbia, Switzerland, the United Kingdom (Scotland, England and Wales) and to two laboratories in South Korea and USA, respectively.
The Belgian NRL covers both Belgium and Luxembourg and the Italian NRL covers Italy and Cyprus for identification of all listed diseases. Figure 3 shows the worldwide distribution of the participating NRLs.

Figure 3. Worldwide distribution of the participants in the EURL proficiency test 2022

Content of ampoules

The viruses were propagated on each of their preferred cell line, and when total cytopathic effect (CPE) was observed, the supernatants were collected and filtrated through a 45 µm filter, mixed with equal volumes of 2% w/v lactalbumin hydrolysate solution and lyophilized in glass ampoules. The ampoules were sealed by melting. The details of the virus isolates used in the proficiency test are outlined in table 1.

Table 1. Content of each ampoule with reference to culture conditions and major publications of the included viruses.

Code	Specifications/References
Ampoule I: IHNV	**IHNV isolate DK 21-4070-1** From farmed rainbow trout in Denmark **Genotype:** E **GenBank accession numbers:** sequence to be submitted in 2023 to NCBI. Available upon request to the EURL
Ampoule II: ECV	**European catfish virus 562/92.** Italian isolate from catfish suffering high mortality. **Received from** Dr. G. Bovo, ISZ-Ve, Padova, Italy. **GenBank accession number**: FJ358608 or KT989884.1 or KT989885.1 or JQ724856.1 **Reference on isolate**: Bovo G, Comuzi M, De Mas S, Ceschia G, Giorgetti G, Giacometti P & Cappellozza E (1993). Isolamento di un agente virale irido-like da pesce gatto (Ictalurus melas) dallevamento. Bollettino Societa Italiana di Patologia Ittica 11, 3–10. **Reference on sequence**: Holopainen R., Ohlemeyer S., Schütze H., Bergmann S.M. & Tapiovaara H. (2009) Ranavirus phylogeny and differentiation based on major capsid protein, DNA polymerase and neurofilament triplet H1-like protein genes. *Diseases of Aquatic Organisms* 85, 81-91.
Ampoule III: SVCV	**SVCV Isolate DK-203273** Spring Viraemia of Carp Virus isolated from Koi Carp in Denmark June 2003 **Genotype: 1a** **Received from:** National Veterinary Institute, Technical University of Denmark. **GenBank accession numbers:** MN094793
Ampoule IV: VHSV	**VHSV NO-2007-50-385** VHSV isolate from sea farmed Rainbow trout in Norway

Code	Specifications/References
	Received from Norwegian Veterinary Institute **Genotype:** IIIb **Ref on isolate:** Dale OB, Ørpetveit I, Lyngstad TM, Kahns S, Skall HF, Olesen NJ, Dannevig BH (2009) Outbreak of viral haemorrhagic septicaemia (VHS) in seawater-farmed rainbow trout in Norway caused by VHS virus Genotype III. Dis Aquat Org 85:93-103. **GenBank accession numbers: G gene:** EU547740 ; **Full genome:** MT162436.1 **Reference on sequence:** Ito, T., Kurita, J., Mori, Ki. et al. Virulence of viral haemorrhagic septicaemia virus (VHSV) genotype III in rainbow trout. Vet Res 47, 4 (2016). https://doi.org/10.1186/s13567-015-0303-z
Ampoule V: IHNV	**IHNV isolate DK 21-4070-1** From farmed rainbow trout in Denmark **Genotype:** E **GenBank accession numbers:** sequence to be submitted in 2023 to NCBI. Available upon request to the EURL

Testing of the PT1 test

The PT1 test was prepared and tested according to protocols accredited under DS/EN ISO/IEC 17043. Prior to distribution the EURL tested 5 ampoules of each virus preparation by titration in 4 cell lines (BF-2, EPC, RTG-2 and FHM), to ascertain a satisfactory titre in the preferred cell line and homogeneity of content of ampoules.

The lyophilisation procedure is known to determine some reduction in the viral titre especially for VHSV. Previous experience reported during the past Proficiency tests demonstrated a rather high stability for SVCV, EHNV and IPNV serotype Sp. Lyophilised viral supernatant mixed in freeze drying medium preserved in glass sealed ampoules is stable for more than half a year when kept at room temperature (Inter-Laboratory Proficiency Test report 2007); it can survive exposure to 30°C for 24 hours (Inter-Laboratory Proficiency Test report 2010) And a temperature raise from 20 to 42°C over a period of 5 hours (Inter-Laboratory Proficiency Test 2011)

The identities of the viruses in all 5 ampoules were checked and confirmed before shipment by ELISA, IFAT, PCR and/or qPCR and RT-PCR and/or RT-qPCR. After shipment the stability of the content in the ampoules were assessed by titrating the virus on cell cultures, and identifying it by ELISA, furthermore PCR based tests were performed on the original content of all the ampoules. This year reductions of the titres after lyophilisation were observed. For all of the ampoules, the reduction of the titre was between 1-2 log in the same cell line. No significant reductions were observed after long term storage (Table 2 and figure 4).

Ampoul No.	Cell line	Titre before Lyophilisation	Titre after Lyophilisation and before shipment	Titre after deadline for handling in results (min. 120 days of storage 4°C in the dark)
		$TCID_{50}$/ml	$TCID_{50}$/ml	$TCID_{50}$/ml
Ampoule I: IHNV DK 21-4070-1	BF-2	8.6E+06	1.3E+05	2.7E+04
	RTG-2	2.7E+07	1.9E+06	1.3E+06
	EPC	4.0E+07	4.0E+06	8.6E+05
	FHM	4.0E+07	2.7E+06	1.9E+05
Ampoule II: ECV Isolate 562/92	BF-2	8.6E+03	4.0E+03	2.7E+03
	RTG-2	4.0E+03	1.3E+03	4.0E+02
	EPC	8.6E+03	8.6E+02	< 1,9E+02
	FHM	< 1,9E+02	< 1,9E+02	< 1,9E+02
Ampoule III: SVCV DK-203273	BF-2	1.9E+07	1.3E+06	8.6E+05
	RTG-2	1.9E+04	5.9E+03	1.9E+04
	EPC	1.9E+07	5.9E+05	1.9E+05
	FHM	4.0E+07	1.3E+06	1.3E+06
Ampoule IV: VHSV NO-2007-50-385	BF-2	2.7E+05	1.9E+04	8.6E+03
	RTG-2	8.6E+05	1.3E+05	8.6E+04
	EPC	1.3E+06	1.3E+05	4.0E+05
	FHM	4.0E+05	1.9E+05	5.9E+04
Ampoule V: IHNV DK 21-4070-1	BF-2	1.9E+02	1.9E+02	1.9E+02
	RTG-2	4.0E+03	4.0E+02	4.0E+02
	EPC	2.7E+03	8.6E+02	1.9E+02
	FHM	1.9E+03	4.0E+02	< 1,9E+02

Table 2. PT1:

Titres in ampoules I to V stored in the dark tested on four cell lines at different time points:

- Before lyophilisation, (stored at -80°C).
- After lyophilisation and before shipment (median titre of 5 replicates), (stored at 4°C), the variation of the titre of the 5 replicates was within 1 log in the same cell line.
- After deadline for handling in results approx. 3 months after shipment (1 ampoule), (stored at 4°C).

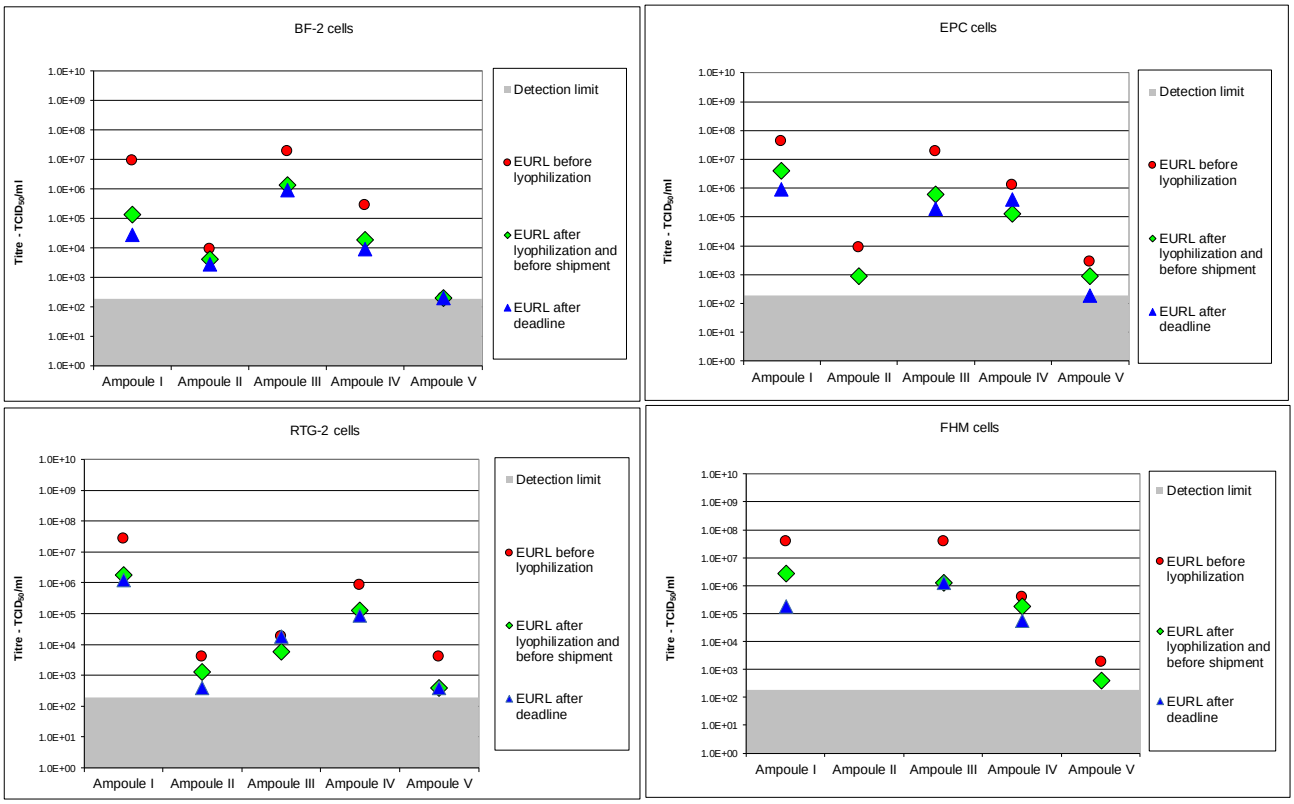


Figure 4. Virus titres in different cell lines: before lyophilisation, before shipment and after deadline for handling in results (storage 4°C in the dark).

Virus identification and titration

Participants were asked to identify the content of each ampoule by the methods used in their laboratory which should be according to the procedures described in the EURL diagnostic manuals [2], i.e. by cell culture followed by ELISA, IFAT and/or RT-PCR/RT-qPCR. The results of the content in the 5 ampoules as reported by the participating laboratories are summarised in table 3.

Participants were also asked to assess the viral load in the ampoules by conducting titrations. The titration procedures were described in the instructions enclosed with the test. All titres were calculated by the EURL based on the crude data submitted by each participant and given as Tissue Culture Infective Dose 50% per ml ($TCID_{50}$/ml). The titre of the re-dissolved virus was multiplied by a factor of 10 to compensate for the dilution of the original volume of virus in the ampoules (200 µl virus + 200 µl lactalbumin in vials re-dissolved in a total of 2.0 ml cell culture medium). The titration results obtained by the participating laboratories are summarised in tables 4 to 8. The titres obtained from each participating laboratory are represented graphically. In Figures 5-8, all titres submitted by the participants for each cell line and ampoule, respectively are compared to each other. On these figures, the median titre and the 25% and 75% inter-quartile range is displayed, the optimal titre will be within these quartiles. A low titer, below 25% quartile may be indicator of low sensitivity of the cell culture in use; conversely a very high titer, beyond 75% quartile may indicate errors in assessing CPE. The titres obtained by each laboratory are plotted in relation to the combined submitted data set and each participating laboratory should be able to compare the sensitivity of their cell lines to the sensitivity of those used by the other participating laboratories. CHSE-214 cells are not displayed graphically or commented on in this report as only 7 laboratories used these cells.
Laboratories were encouraged to identify the genotype of the virus isolates.

It was requested that the pathogens in the samples were not forwarded to third parts without having contacted the EURL for permission in advance.

Table 3. Inter-Laboratory Proficiency Test, PT1, 2022 - Virus identification and score obtained by participants.

Laboratory code number	Score	Ampoule I	Ampoule II	Ampoule III	Ampoule IV	Ampoule V
		IHNV DK 21-4070-1	**ECV 562/92**	**SVCV DK-203273**	**VHSV NO-2007-50-385**	**IHNV DK 21-4070-1**
1	10/10	IHNV	RANAvirus, not EHNV	SVCV	VHSV	IHNV
2	10/10	IHNV	Ranavirus, no EHNV	SVCV	VHSV	IHNV
3	10/10	IHNV	Ranavirus/European catfish virus	SVCV	VHSV	IHNV
4	10/10	IHNV	Ranavirus - NOT EHNV	SVCV	VHSV	IHNV
5	10/10	IHNV	ECV	SVCV	VHSV	IHNV
6	10/10	IHNV	Ranavirus-NOT EHNV	SVCV	VHSV	IHNV
7	8/10	IHNV	Ranavirus	SVCV	VHSV	NO VHSV,NO IHNV,NO EHNV,NO RANAVIRUS ,NO IPNV,NO SVCV
8	8/10	IHNV	European Catfish Virus	SVCV	VHSV	0
9	10/10	IHNV	Ranavirus-NOT EHNV	SVCV	VHSV	IHNV
10	10/10	IHNV	Ranavirus - Not EHNV	SVCV	VHSV	IHNV
11	10/10	IHN	Ranavirus – NOT EHNV	SVCV	VHS	IHN
12	10/10	IHNV	Ranavirus	SVCV	VHSV	IHNV
13	10/10	IHNV	Ranavirus - NOT EHNV	SVCV	VHSV	IHNV
14	8/10	IHNV	NEGATIVE	SVCV	VHSV	IHNV
15	10/10	IHNV	Ranavirus	SVCV	VHSV	IHNV
16	10/10	IHNV	Ranavirus - NOT EHNV	SVCV	VHSV	IHNV
17	10/10	IHNV	Ranavirus - not EHNV	SVCV	VHSV	IHNV
18	8/8	IHNV	Ranavirus – NOT EHNV	Negative for IHNV, Ranavirus and VHSV	VHSV	IHNV
19	9/10	IHNV	Ranavirus, Not EHNV	SVCV, IHNV	VHSV	IHNV
20	10/10	IHNV	Ranavirus-NOT EHNV	SVCV	VHSV	IHNV
21	10/10	IHNV	Ranavirus – NOT EHNV	SVCV	VHSV	IHNV
22	7/10	IHNV	not EHNV	SVCV, IPNV	VHSV	-
23	8/10	IHNV	Ranavirus -ECV	0	VHSV	IHNV

24	8/10	IHNV	Ranavirus (European Catfish virus)	SVCV	VHSV	Negative
25	8/10	IHNV	Ranavirus	SVCV	VHSV	-
26	10/10	IHN	"Ranavirus NOT EHNV"	SVC	VHS	IHN
27	10/10	IHNV	Ranavirus - NOT EHNV	SVCV	VHSV	IHNV
28	10/10	IHNV	ECV/ESV	SVCV	VHSV	IHNV
29	10/10	IHNV	Ranavirus – NOT EHNV	SVCV	VHSV	IHNV
30	10/10	IHNV	Ranavirus - NOT EHNV	SVCV	VHSV	IHNV
31	10/10	IHNV	Ranavirus - European sheatfish virus	SVCV	VHSV	IHNV
32	10/10	IHNV	Ranavirus – NOT EHNV	SVCV	VHSV	IHNV
33	10/10	IHNV	ECV	SVCV	VHSV	IHNV
34	10/10	IHNV	Ranavirus - Not EHNV	SVCV	VHSV	IHNV
35	10/10	IHNV	Ranavirus - NOT EHNV	SVCV	VHSV	IHNV
36	8/10	IHNV	EHNV	SVCV	VHSV	IHNV
37	10/10	IHNV	Rana Not EHNV	SVCV	VHSV	IHNV
38	8/8	IHNV	NO IHNV, VHSV,SVCV, IPNV	SVCV	VHSV	IHNV
39	8/10	IHNV	Ranavirus	SVCV	VHSV	IHNV
40	10/10	IHNV	Ranavirus - NOT EHNV	SVCV	VHSV	IHNV
41	10/10	IHNV	ECV	SVCV	VHSV	IHNV
42	10/10	IHNV	Ranavirus - not EHNV	SVCV	VHSV	IHNV

1) Do not test for Ranavirus

2) Did not corroborate the findings in ampoule II by sequencing or REA or have used commercial qPCR kit distinguish between EHNV and other Ranavirus

3) Do not test for SVCV

Table 4. Inter-Laboratory Proficiency Test, PT1, 2022 – **Results of titration of ampoule I.**

Laboratory Code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
1	IHNV	2.7E+04	4.0E+06	2.7E+06	4.0E+06
2	IHNV	5.9E+05	1.9E+06	N/A	N/A
3	IHNV	N/A	N/A	N/A	N/A
4	IHNV	8.6E+03	4.0E+05	N/A	N/A
5	IHNV	8.6E+03	2.7E+06	8.6E+05	N/A
6	IHNV	1.9E+04	5.9E+06	4.0E+05	N/A
7	IHNV	2.7E+04	1.3E+05	N/A	N/A
8	IHNV	1.9E+03	8.6E+04	N/A	N/A
9	IHNV	2.7E+05	2.7E+06	N/A	N/A
10	IHNV	5.9E+03	1.9E+06	N/A	N/A
11	IHN	5.9E+04	5.9E+05	N/A	N/A
12	IHNV	<1,9E+02	4.0E+05	4.0E+04	1.9E+06
13	IHNV	8.6E+03	2.7E+05	N/A	N/A
14	IHNV	N/A	8.6E+05	4.0E+05	N/A
15	IHNV	1.3E+05	8.6E+05	N/A	N/A
16	IHNV	1.3E+03	8.6E+05	N/A	N/A
17	IHNV	2.7E+03	1.3E+06	N/A	1.9E+06
18	IHNV	N/A	N/A	N/A	N/A
19	IHNV	N/A	N/A	N/A	N/A
20	IHNV	8.6E+04	N/A	N/A	N/A
21	IHNV	2.7E+07	4.0E+07	N/A	N/A
22	IHNV	5.9E+02	5.9E+02	4.0E+02	N/A
23	IHNV	<1,9E+02	1.9E+07	N/A	N/A
24	IHNV	2.7E+02	8.6E+04	4.0E+02	5.9E+03
25	IHNV	N/A	5.9E+05	N/A	2.7E+04
26	IHN	1.3E+05	1.3E+06	1.3E+04	8.6E+05
27	IHNV	2.7E+04	N/A	N/A	2.7E+03
28	IHNV	2.7E+05	2.7E+07	N/A	N/A
29	IHNV	1.9E+04	N/A	N/A	5.9E+04
30	IHNV	5.9E+03	8.6E+06	N/A	N/A
31	IHNV	N/A	5.9E+05	N/A	1.3E+06
32	IHNV	2.7E+04	2.7E+06	N/A	N/A
33	IHNV	1.9E+03	1.9E+05	1.3E+05	N/A
34	IHNV	2.7E+04	5.9E+04	N/A	5.9E+05
35	IHNV	1.3E+04	5.9E+05	1.3E+05	N/A
36	IHNV	4.0E+04	5.9E+06	N/A	N/A
37	IHNV	< 1,9E+02	1.9E+07	4.0E+05	8.6E+05
38	IHNV	2.7E+04	4.0E+05	N/A	N/A
39	IHNV	5.9E+03	8.6E+05	N/A	N/A
40	IHNV	1.3E+05	5.9E+05	8.6E+03	1.3E+06
41	IHNV	2.7E+04	8.6E+07	N/A	N/A
42	IHNV	1.3E+03	8.6E+05	N/A	1.3E+06

N/A: Cell line not applied by the participating laboratory for titration of the virus

IHNV DK 21-4070-1	BF-2	EPC	RTG-2	FHM
Number of laboratories	36	36	12	13
Median titre	1.9E+04	8.6E+05	1.3E+05	8.6E+05
Maximum titre	2.7E+07	8.6E+07	2.7E+06	4.0E+06
Minimum titre	<1,9E+02	5.9E+02	4.0E+02	2.7E+03
25% quartile titre	2.5E+03	4.0E+05	1.2E+04	5.9E+04
75% quartile titre	4.5E+04	3.0E+06	4.0E+05	1.3E+06

Table 5. Inter-Laboratory Proficiency Test, PT1, 2022 – **Results of titration of ampoule II.**

Laboratory Code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
1	RANAvirus, not EHNV	5.9E+03	2.7E+02	1.3E+03	<1,9E+02
2	Ranavirus, no EHNV	5.9E+04	2.7E+03	N/A	N/A
3	Ranavirus/European catfish virus		N/A	N/A	N/A
4	Ranavirus - NOT EHNV	4.0E+02	<1,9E+02	N/A	N/A
5	ECV	2.7E+04	5.9E+03	1.9E+03	N/A
6	Ranavirus-NOT EHNV	2.7E+03	1.9E+03	8.6E+02	N/A
7	Ranavirus	2.7E+03	8.6E+02	N/A	N/A
8	European Catfish Virus	4.0E+03	1.9E+03	N/A	N/A
9	Ranavirus-NOT EHNV	<1,9E+02	<1,9E+02	N/A	N/A
10	Ranavirus - Not EHNV	8.6E+03	5.9E+02	N/A	N/A
11	Ranavirus – NOT EHNV	1.3E+03	<1,9E+02	N/A	N/A
12	Ranavirus	2.7E+04	5.9E+02	<1,9E+02	<1,9E+02
13	Ranavirus - NOT EHNV	<1,9E+02	<1,9E+02	N/A	N/A
14	NEGATIVE	N/A	<1,9E+02	<1,9E+02	N/A
15	Ranavirus	4.0E+04	5.9E+03	N/A	N/A
16	Ranavirus - NOT EHNV	2.7E+03	1.3E+03	N/A	N/A
17	Ranavirus - not EHNV	2.7E+03	1.9E+03	N/A	1.3E+03
18	Ranavirus – NOT EHNV	N/A	N/A	N/A	N/A
19	Ranavirus, Not EHNV	N/A	N/A	N/A	N/A
20	Ranavirus-NOT EHNV	1.3E+03	N/A	N/A	N/A
21	Ranavirus – NOT EHNV	4.0E+06	1.3E+06	N/A	N/A
22	not EHNV	1.3E+04	8.6E+03	8.6E+03	N/A
23	Ranavirus -ECV	1.9E+05	4.0E+04	N/A	N/A
24	Ranavirus (European Catfish virus)	1.3E+03	2.7E+03	4.0E+02	5.9E+02
25	Ranavirus	N/A	1.9E+03	N/A	4.0E+02
26	"Ranavirus NOT EHNV"	1.3E+04	1.3E+03	1.3E+04	1.3E+03
27	Ranavirus - NOT EHNV	1.9E+04	N/A	N/A	<1,9E+02
28	ECV/ESV	<1,9E+02	<1,9E+02	N/A	N/A
29	Ranavirus – NOT EHNV	1.3E+03	N/A	N/A	<1,9E+02
30	Ranavirus - NOT EHNV	8.6E+04	8.6E+03	N/A	N/A
31	Ranavirus - European sheatfish virus	2.7E+03	1.3E+03	N/A	N/A
32	Ranavirus – NOT EHNV	2.7E+04	1.9E+03	N/A	N/A
33	ECV	1.3E+04	1.3E+03	<1,9E+02	N/A
34	Ranavirus - Not EHNV	8.6E+03	8.6E+03	N/A	8.6E+02
35	Ranavirus - NOT EHNV	1.3E+04	1.3E+03	<1,9E+02	N/A
36	EHNV	2.7E+04	5.9E+03	N/A	N/A
37	Rana Not EHNV	1.3E+04	<1,9E+02	<1,9E+02	<1,9E+02
38	NO IHNV, VHSV,SVCV, IPNV	8.6E+03	1.9E+03	N/A	N/A
39	Ranavirus	1.3E+04	4.0E+03	N/A	N/A
40	Ranavirus - NOT EHNV	1.9E+04	1.3E+03	<1,9E+02	<1,9E+02
41	ECV	8.6E+03	8.6E+05	N/A	N/A
42	Ranavirus - not EHNV	2.7E+03	8.6E+03	N/A	5.9E+02

N/A: Cell line not applied by the participating laboratory for titration of the virus

ECV Isolate 562/92	BF-2	EPC	RTG-2	FHM
Number of laboratories	37	36	12	12
Median titre	8.6E+03	1.9E+03	2.0E+02	2.0E+02
Maximum titre	4.0E+06	1.3E+06	1.3E+04	1.3E+03
Minimum titre	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
25% quartile titre	2.7E+03	5.9E+02	<1,9E+02	<1,9E+02
75% quartile titre	1.9E+04	5.9E+03	1.4E+03	6.6E+02

Table 6. Inter-Laboratory Proficiency Test, PT1, 2022 – **Results of titration of ampoule III**

Laboratory Code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
1	SVCV	5.9E+05	1.9E+06	5.9E+04	2.7E+06
2	SVCV	5.9E+05	4.0E+05	N/A	N/A
3	SVCV	N/A	N/A	N/A	N/A
4	SVCV	8.6E+04	1.3E+05	N/A	N/A
5	SVCV	1.3E+06	2.7E+06	8.6E+05	N/A
6	SVCV	2.7E+05	8.6E+05	8.6E+02	N/A
7	SVCV	1.3E+05	1.3E+04	N/A	N/A
8	SVCV	4.0E+03	5.9E+04	N/A	N/A
9	SVCV	5.9E+05	1.9E+05	N/A	N/A
10	SVCV	2.7E+06	5.9E+05	N/A	N/A
11	SVCV	8.6E+04	<1,9E+02	N/A	N/A
12	SVCV	5.9E+02	2.7E+04	<1,9E+02	2.7E+04
13	SVCV	1.3E+04	8.6E+03	N/A	N/A
14	SVCV	N/A	4.0E+05	<1,9E+02	N/A
15	SVCV	4.0E+04	8.6E+04	N/A	N/A
16	SVCV	1.3E+05	4.0E+05	N/A	N/A
17	SVCV	4.0E+03	4.0E+05	N/A	1.3E+06
18	Negative for IHNV, Ranavirus and VHSV	N/A	N/A	N/A	N/A
19	SVCV, IHNV	N/A	N/A	N/A	N/A
20	SVCV	1.9E+04	N/A	N/A	N/A
21	SVCV	5.9E+07	1.3E+08	N/A	N/A
22	SVCV, IPNV	8.6E+02	5.9E+02	8.6E+02	N/A
23	0	<1,9E+02	1.3E+06	N/A	N/A
24	SVCV	1.00E+00	4.00E+03	<1,9E+02	8.62E+03
25	SVCV	N/A	5.87E+03	N/A	1.26E+03
26	SVC	2.7E+04	1.3E+04	4.0E+03	4.0E+04
27	SVCV	1.3E+05	N/A	N/A	2.7E+04
28	SVCV	2.7E+05	2.7E+06	N/A	N/A
29	SVCV	4.0E+04	N/A	N/A	1.3E+05
30	SVCV	1.9E+06	2.7E+05	N/A	N/A
31	SVCV	N/A	5.87E+04	N/A	5.9E+04
32	SVCV	1.3E+06	1.9E+05	N/A	N/A
33	SVCV	1.9E+03	1.3E+05	1.3E+03	N/A
34	SVCV	1.9E+03	1.3E+04	N/A	2.7E+04
35	SVCV	1.3E+04	8.6E+03	<1,9E+02	N/A
36	SVCV	5.9E+04	8.6E+05	N/A	N/A
37	SVCV	2.7E+03	4.0E+05	<1,9E+02	2.7E+02
38	SVCV	4.00E+04	4.00E+03	N/A	N/A
39	SVCV	2.7E+04	1.3E+05	N/A	N/A
40	SVCV	1.3E+04	1.9E+05	<1,9E+02	2.7E+05
41	SVCV	1.9E+05	2.7E+05	N/A	N/A
42	SVCV	5.9E+04	5.9E+04	N/A	5.9E+04

N/A: Cell line not applied by the participating laboratory for titration of the virus

SVCV DK-203273	BF-2	EPC	RTG-2	FHM
Number of laboratories	36	36	12	13
Median titre	4.9E+04	1.6E+05	4.3E+02	4.0E+04
Maximum titre	5.9E+07	1.3E+08	8.6E+05	2.7E+06
Minimum titre	<1,9E+02	<1,9E+02	<1,9E+02	2.7E+02
25% quartile titre	1.0E+04	1.3E+04	<1,9E+02	2.7E+04
75% quartile titre	2.7E+05	4.0E+05	1.9E+03	1.3E+05

Table 7. Inter-Laboratory Proficiency Test, PT1, 2022 – **Results of titration of ampoule IV.**

Laboratory Code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
1	VHSV	8.6E+02	2.7E+05	4.0E+04	2.7E+05
2	VHSV	4.0E+03	4.0E+04	N/A	N/A
3	VHSV	N/A	N/A	N/A	N/A
4	VHSV	1.9E+03	2.7E+04	N/A	N/A
5	VHSV	1.3E+04	8.6E+04	2.7E+04	N/A
6	VHSV	1.3E+04	1.9E+05	1.3E+04	N/A
7	VHSV	1.3E+03	5.9E+03	N/A	N/A
8	VHSV	8.6E+02	1.3E+03	N/A	N/A
9	VHSV	8.6E+03	1.9E+04	N/A	N/A
10	VHSV	5.9E+03	8.6E+04	N/A	N/A
11	VHS	1.9E+03	4.0E+04	N/A	N/A
12	VHSV	1.3E+03	8.6E+03	8.6E+03	8.6E+04
13	VHSV	4.0E+03	5.9E+04	N/A	N/A
14	VHSV	N/A	1.3E+05	4.0E+03	N/A
15	VHSV	2.7E+03	4.0E+04	N/A	N/A
16	VHSV	4.0E+03	8.6E+04	N/A	N/A
17	VHSV	2.7E+03	1.3E+05	N/A	5.9E+04
18	VHSV	N/A	N/A	N/A	N/A
19	VHSV	N/A	N/A	N/A	N/A
20	VHSV	1.3E+03	N/A	N/A	N/A
21	VHSV	4.0E+07	1.9E+07	N/A	N/A
22	VHSV	1.9E+02	2.7E+02	2.7E+02	N/A
23	VHSV	<1,9E+02	4.0E+04	N/A	N/A
24	VHSV	<1,9E+02	1.3E+03	<1,9E+02	4.0E+03
25	VHSV	N/A	1.3E+03	N/A	1.3E+04
26	VHS	4.0E+03	8.6E+04	1.9E+04	8.6E+04
27	VHSV	5.9E+03	N/A	N/A	1.9E+03
28	VHSV	8.6E+04	5.9E+05	N/A	N/A
29	VHSV	2.7E+04	N/A	N/A	1.3E+04
30	VHSV	1.3E+04	1.9E+05	N/A	N/A
31	VHSV	2.7E+04	4.0E+04	N/A	N/A
32	VHSV	4.0E+03	5.9E+04	N/A	N/A
33	VHSV	1.3E+03	5.9E+03	4.0E+02	N/A
34	VHSV	4.0E+02	1.3E+04	N/A	1.9E+04
35	VHSV	1.9E+03	4.0E+04	1.3E+03	N/A
36	VHSV	2.7E+03	5.9E+04	N/A	N/A
37	VHSV	<1,9E+02	2.7E+04	2.7E+02	2.7E+03
38	VHSV	1.9E+04	8.6E+04	N/A	N/A
39	VHSV	4.0E+04	1.9E+05	N/A	N/A
40	VHSV	1.3E+04	1.3E+04	<1,9E+02	4.0E+03
41	VHSV	1.9E+04	5.9E+05	N/A	N/A
42	VHSV	1.9E+04	5.9E+04	N/A	4.0E+04

N/A: Cell line not applied by the participating laboratory for titration of the virus

VHSV NO-2007-50-385	BF-2	EPC	RTG-2	FHM
Number of laboratories	37	36	12	12
Median titre	4.0E+03	4.9E+04	2.6E+03	1.6E+04
Maximum titre	4.0E+07	1.9E+07	4.0E+04	2.7E+05
Minimum titre	<1,9E+02	2.7E+02	<1,9E+02	1.9E+03
25% quartile titre	1.3E+03	1.7E+04	2.7E+02	4.0E+03
75% quartile titre	1.3E+04	9.6E+04	1.4E+04	6.6E+04

Table 8. Inter-Laboratory Proficiency Test, PT1, 2022 – Results of titration of **ampoule V**.

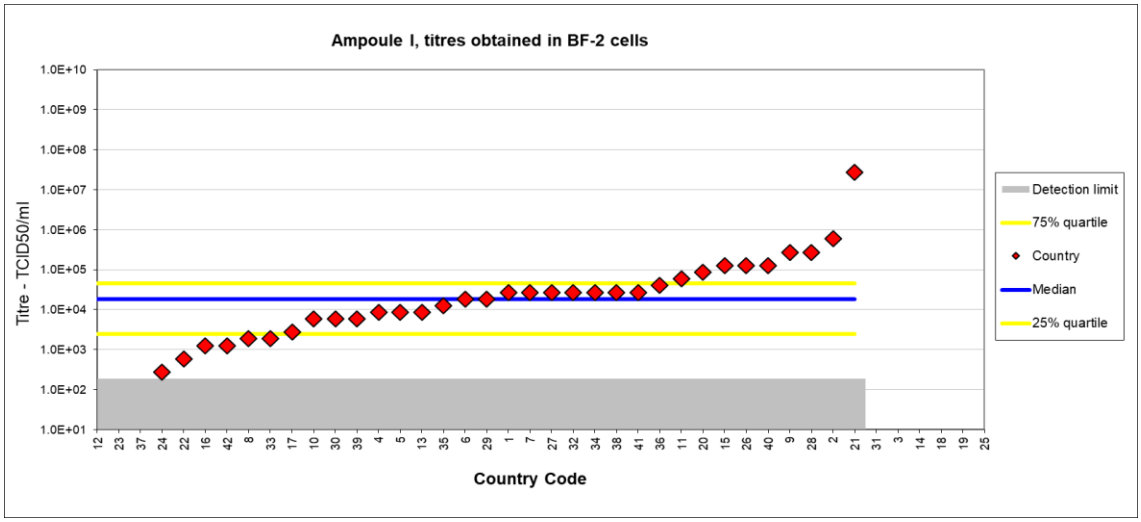
Laboratory Code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
1	IHNV	< 1,9E+02	1.9E+03	1.3E+03	8.6E+02
2	IHNV	2.7E+02	4.0E+04	N/A	N/A
3	IHNV	N/A	N/A	N/A	N/A
4	IHNV	< 1,9E+02	1.3E+03	N/A	N/A
5	IHNV	< 1,9E+02	1.3E+03	< 1,9E+02	N/A
6	IHNV	< 1,9E+02	8.6E+02	1.3E+03	N/A
7	NO VHSV,NO IHNV,NO EHNV,NO RANAVIRUS ,NO IPNV,NO SVCV	< 1,9E+02	< 1,9E+02	N/A	N/A
8	0	< 1,9E+02	< 1,9E+02	N/A	N/A
9	IHNV	< 1,9E+02	1.9E+03	N/A	N/A
10	IHNV	< 1,9E+02	5.9E+02	N/A	N/A
11	IHN	1.9E+02	5.9E+02	N/A	N/A
12	IHNV	< 1,9E+02	4.0E+02	1.9E+02	8.6E+02
13	IHNV	< 1,9E+02	4.0E+02	N/A	N/A
14	IHNV	N/A	1.3E+03	< 1,9E+02	N/A
15	IHNV	1.3E+04	2.7E+02	N/A	N/A
16	IHNV	< 1,9E+02	1.3E+03	N/A	N/A
17	IHNV	1.3E+03	1.9E+03	N/A	1.9E+03
18	IHNV	N/A	N/A	N/A	N/A
19	IHNV	N/A	N/A	N/A	N/A
20	IHNV	1.3E+03	N/A	N/A	N/A
21	IHNV	2.7E+05	1.9E+05	N/A	N/A
22	-	1.9E+02	1.9E+02	< 1,9E+02	N/A
23	IHNV	< 1,9E+02	5.9E+03	N/A	N/A
24	Negative	< 1,9E+02	< 1,9E+02	< 1,9E+02	< 1,9E+02
25	-	N/A	< 1,9E+02	N/A	< 1,9E+02
26	IHN	1.3E+03	1.3E+03	1.3E+03	1.9E+03
27	IHNV	1.9E+02	N/A	N/A	1.9E+02
28	IHNV	< 1,9E+02	1.3E+03	N/A	N/A
29	IHNV	< 1,9E+02	N/A	N/A	8.6E+02
30	IHNV	< 1,9E+02	2.7E+03	N/A	N/A
31	IHNV	N/A	5.9E+02	N/A	1.3E+03
32	IHNV	< 1,9E+02	4.0E+03	N/A	N/A
33	IHNV	1.9E+02	5.9E+02	5.9E+02	N/A
34	IHNV	2.7E+02	8.6E+02	N/A	1.9E+03
35	IHNV	< 1,9E+02	1.3E+03	2.7E+02	N/A
36	IHNV	< 1,9E+02	5.9E+03	N/A	N/A
37	IHNV	< 1,9E+02	4.0E+03	4.0E+02	4.0E+02
38	IHNV	1.9E+02	2.7E+02	N/A	N/A
39	IHNV	5.9E+03	1.3E+06	N/A	N/A

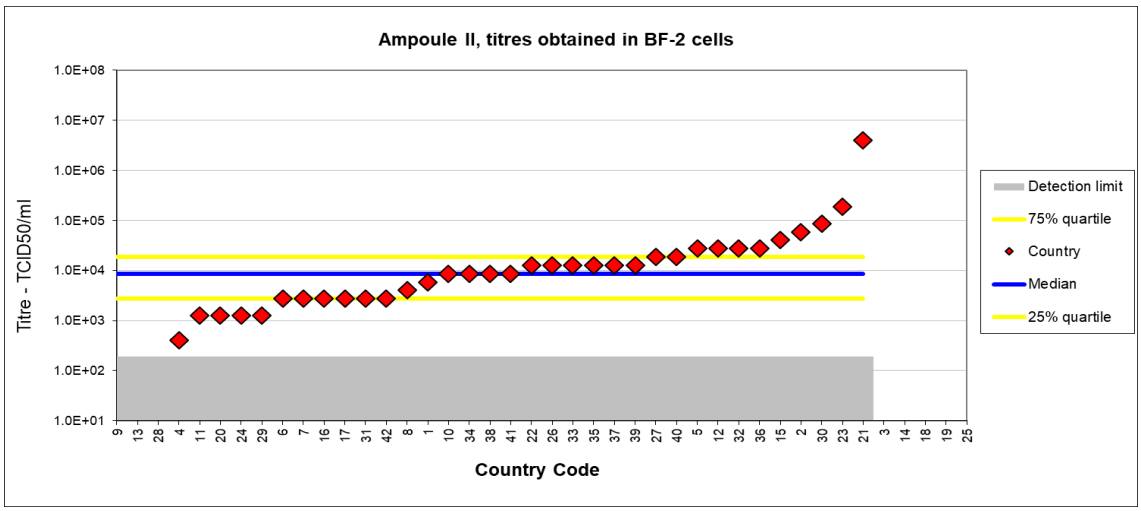
40	IHNV	< 1,9E+02	5.9E+02	< 1,9E+02	1.9E+02
41	IHNV	< 1,9E+02	1.3E+04	N/A	N/A
42	IHNV	1.3E+03	1.3E+03	N/A	8.6E+02

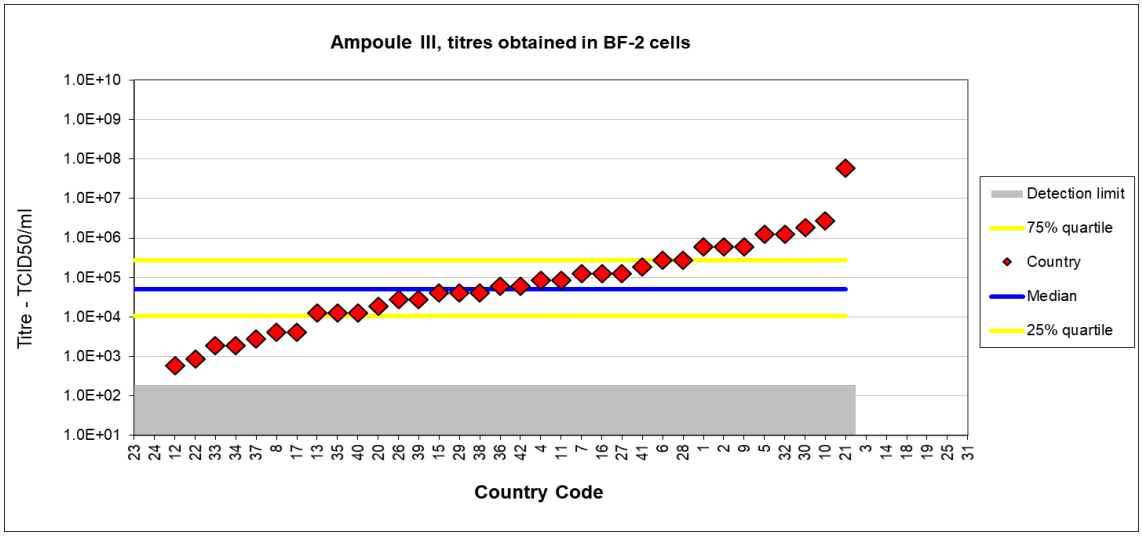
N/A: Cell line not applied by the participating laboratory for titration of the virus

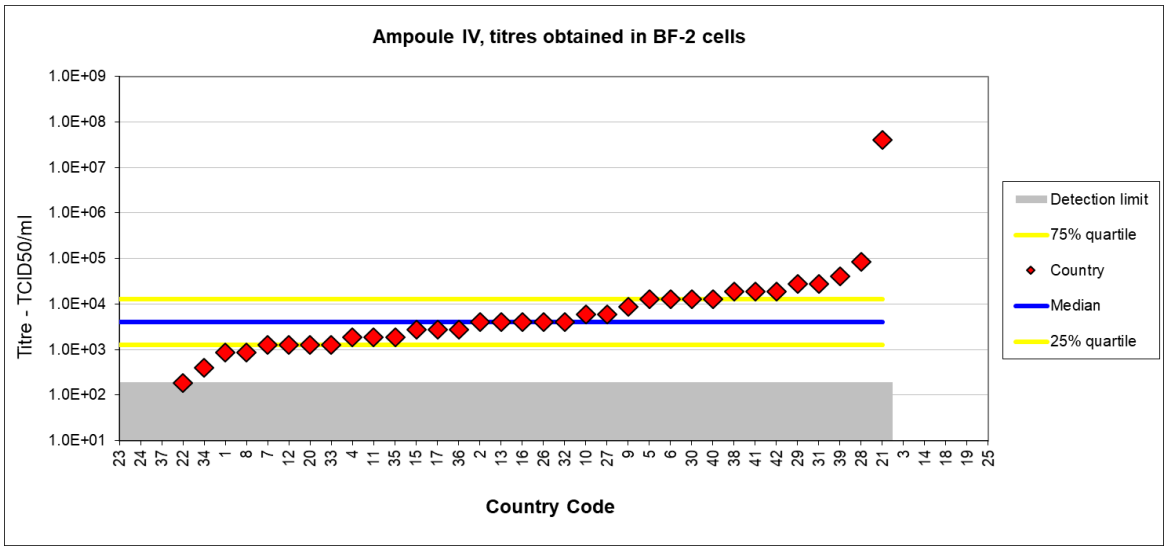
IHNV DK 21-4070-1	**BF-2**	**EPC**	**RTG-2**	**FHM**
Number of laboratories	36	36	12	13
Median titre	<1,9E+02	1.3E+03	2.3E+02	8.6E+02
Maximum titre	2.7E+05	1.3E+06	1.3E+03	1.9E+03
Minimum titre	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
25% quartile titre	<1,9E+02	5.4E+02	<1,9E+02	1.9E+02
75% quartile titre	2.1E+02	2.1E+03	7.6E+02	1.3E+03

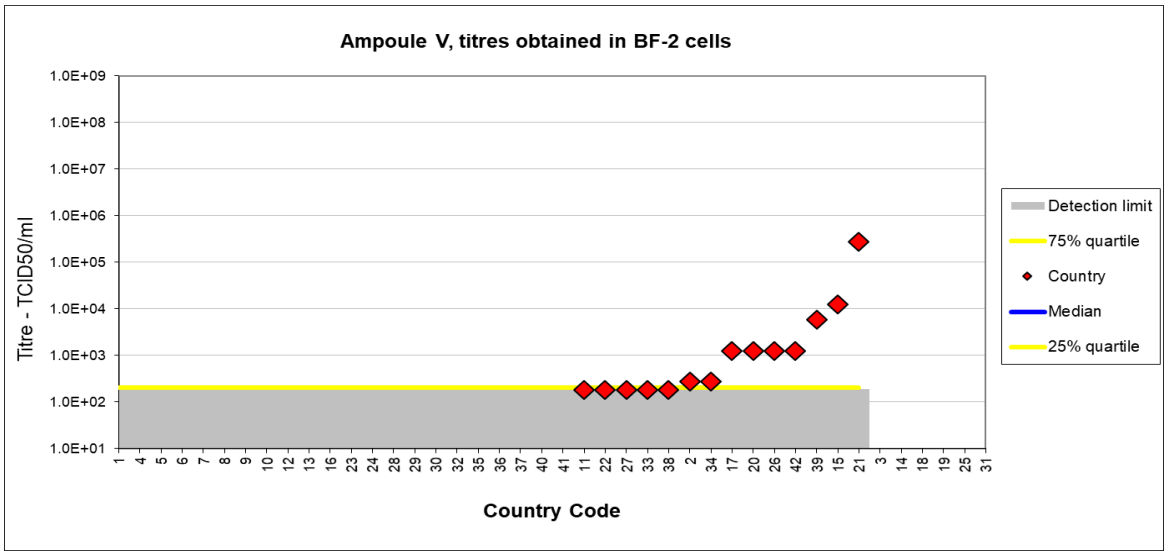
Figure 5. Virus titres obtained in BF-2 cells. The titre (red diamond) of each participating laboratory (country code on the x axis) using BF-2 cells illustrated for ampoule I, II, III, IV and V. The detection limit (grey shadow), median (blue line), 75% quartile (upper yellow line) and 25% quartile (lower yellow line) are plotted on all graphs. Participants failing to obtain any titre are listed on the x axis under the grey zone but no red diamond is plotted; participants who did not use a specific cell line are listed after the the grey zone.





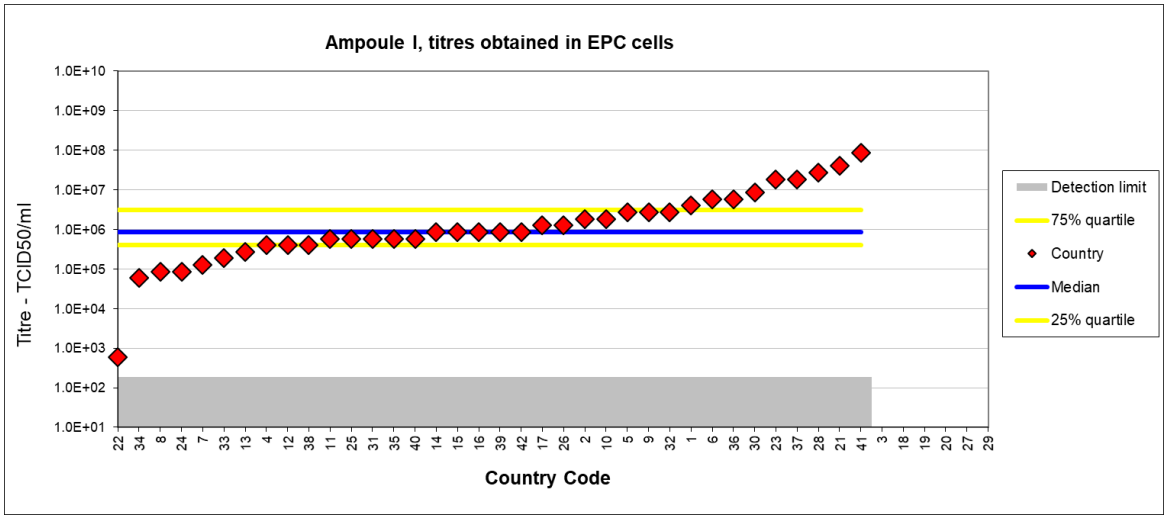


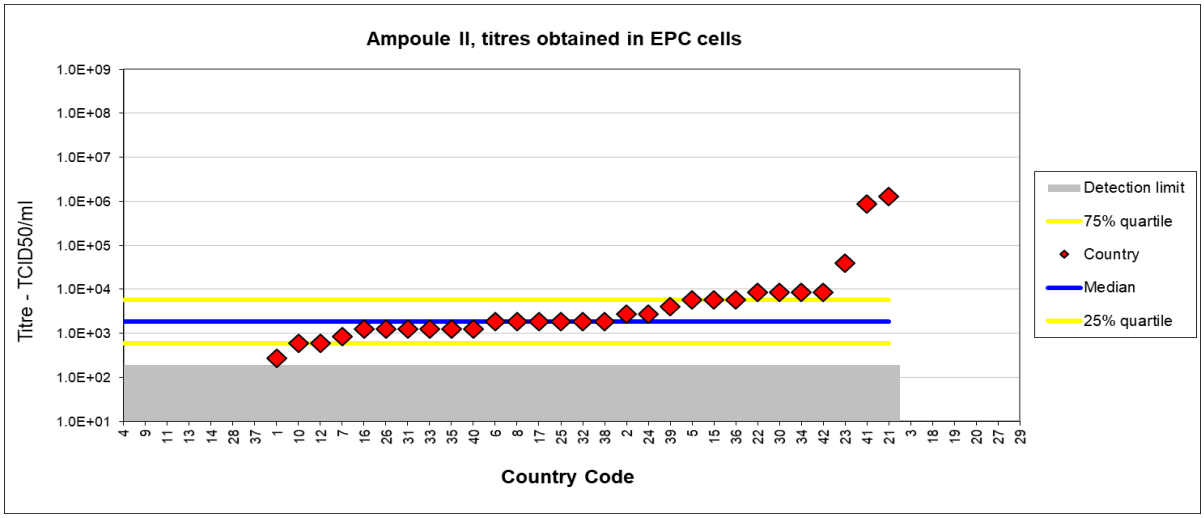


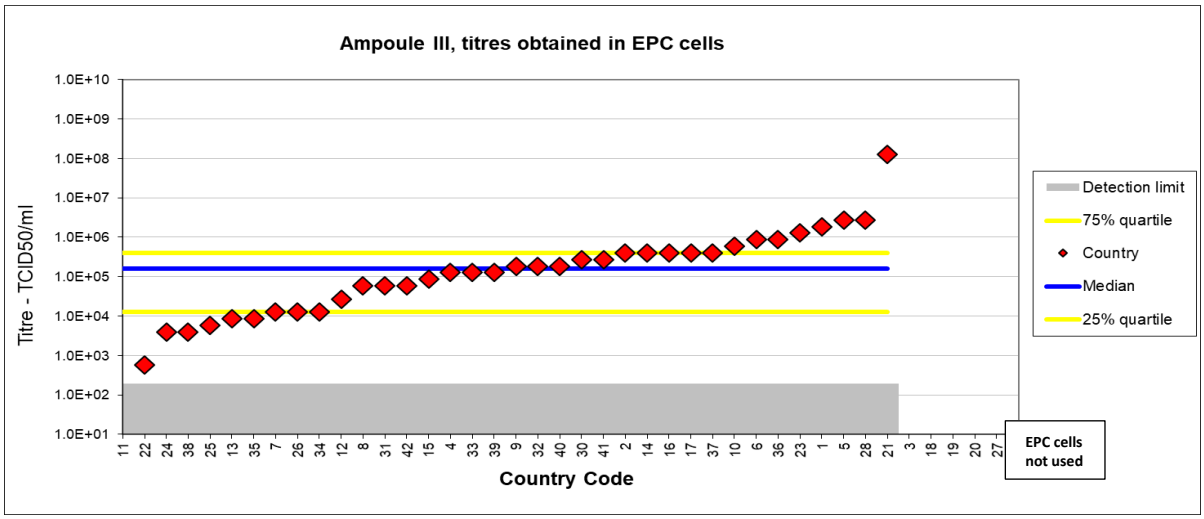


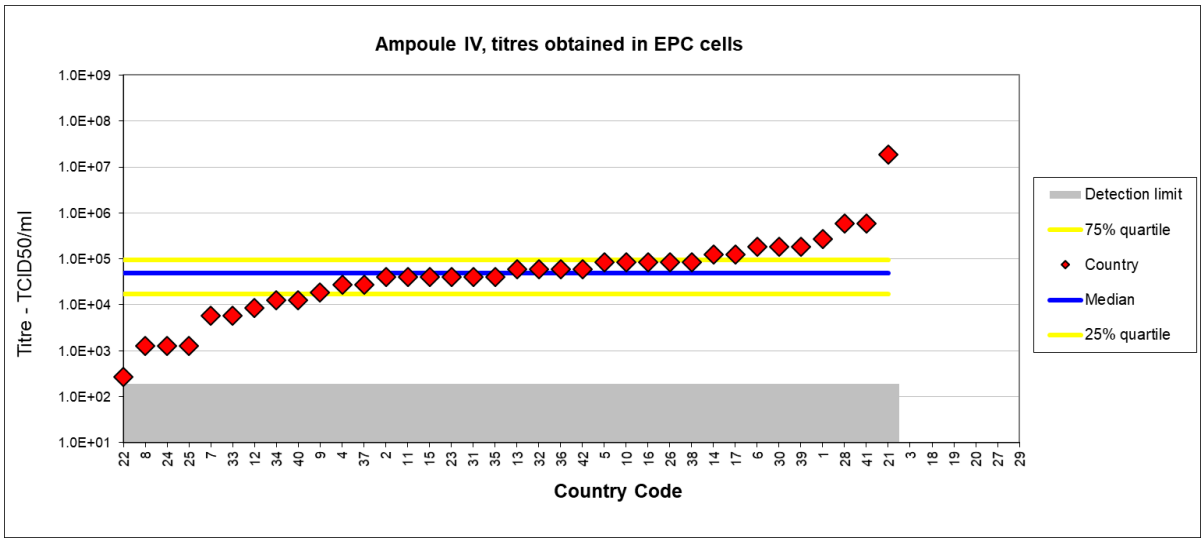
Median (blue line) and 25% quartile (lower yellow line) are under the detection limit (grey shadow)

Figure 6. Virus titres obtained in EPC cells. For further details see description at Figure 5








Ampoule IV, titres obtained in EPC cells
Titre - TCID50/ml
1.0E+09
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
22 8 24 25 7 33 12 34 40 9 4 37 2 11 15 23 31 35 13 32 36 42 5 10 16 26 38 14 17 6 30 39 1 28 41 21 3 18 19 20 27 29
Country Code
Detection limit
75% quartile
Country
Median
25% quartile

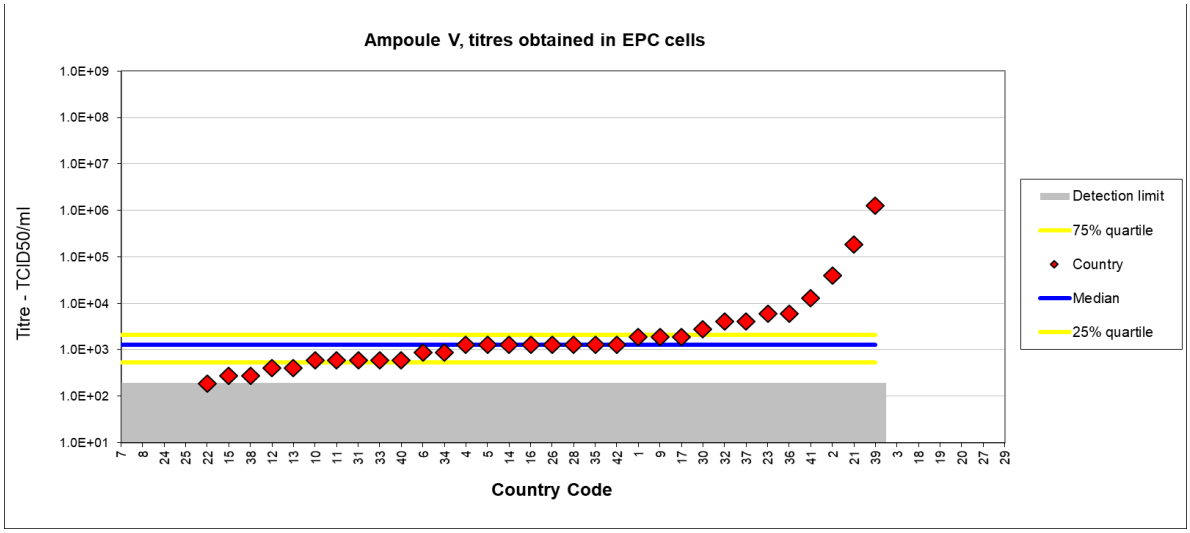
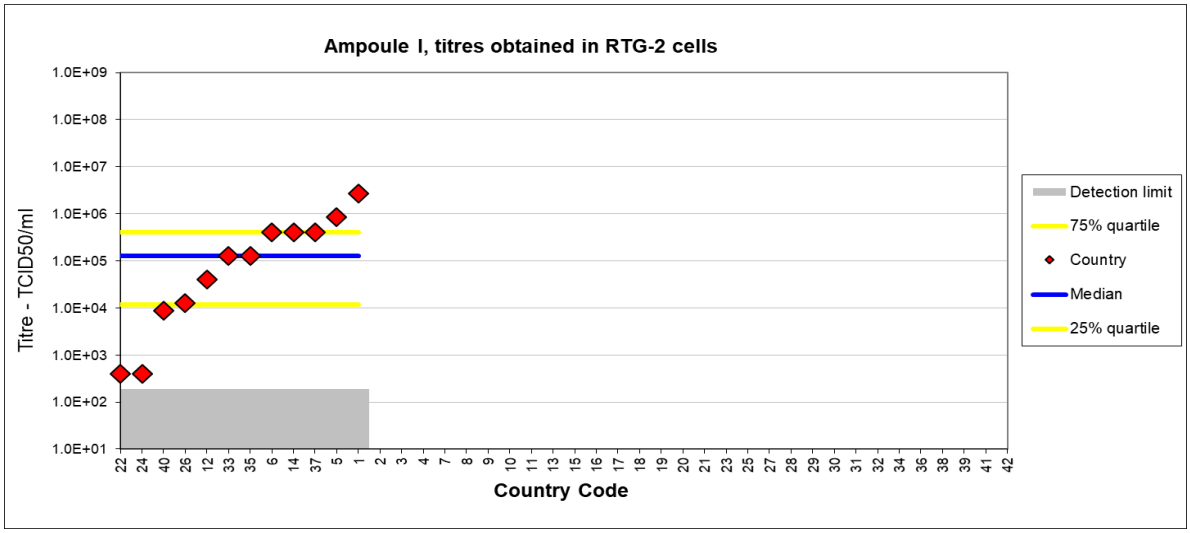
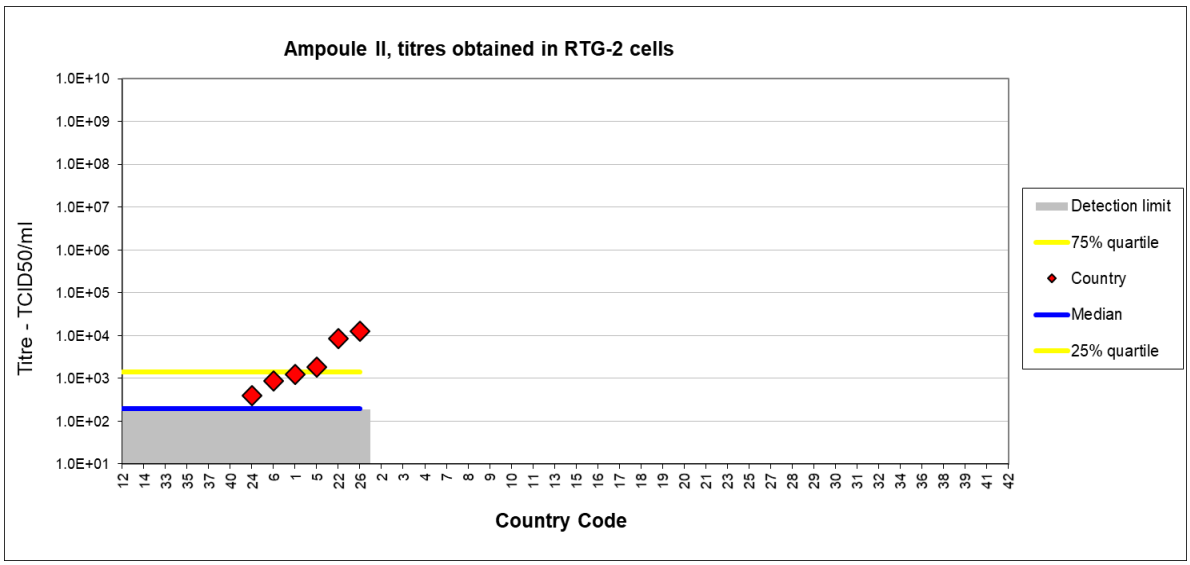
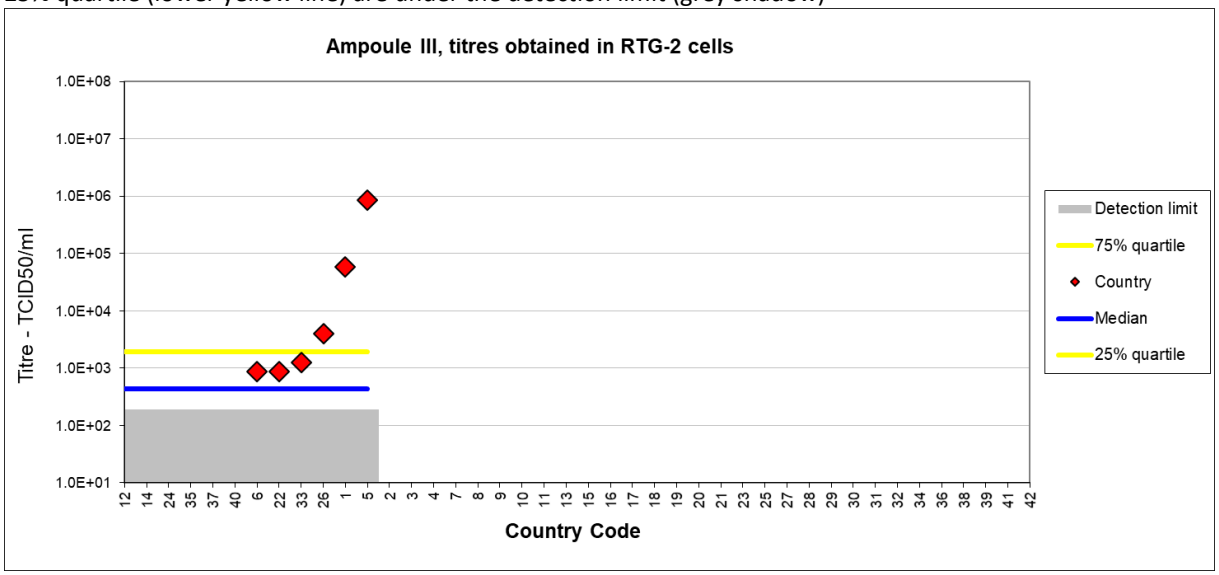

Ampoule V, titres obtained in EPC cells
Titre - TCID50/ml
1.0E+09
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
7 8 24 25 22 15 38 12 13 10 11 31 33 40 6 34 4 5 14 16 26 28 35 42 1 9 17 30 32 37 23 36 41 2 21 39 3 18 19 20 27 29
Country Code
Detection limit
75% quartile
Country
Median
25% quartile

Figure 7. Virus titre obtained in RTG-2 cells. For further details see description at Figure 5

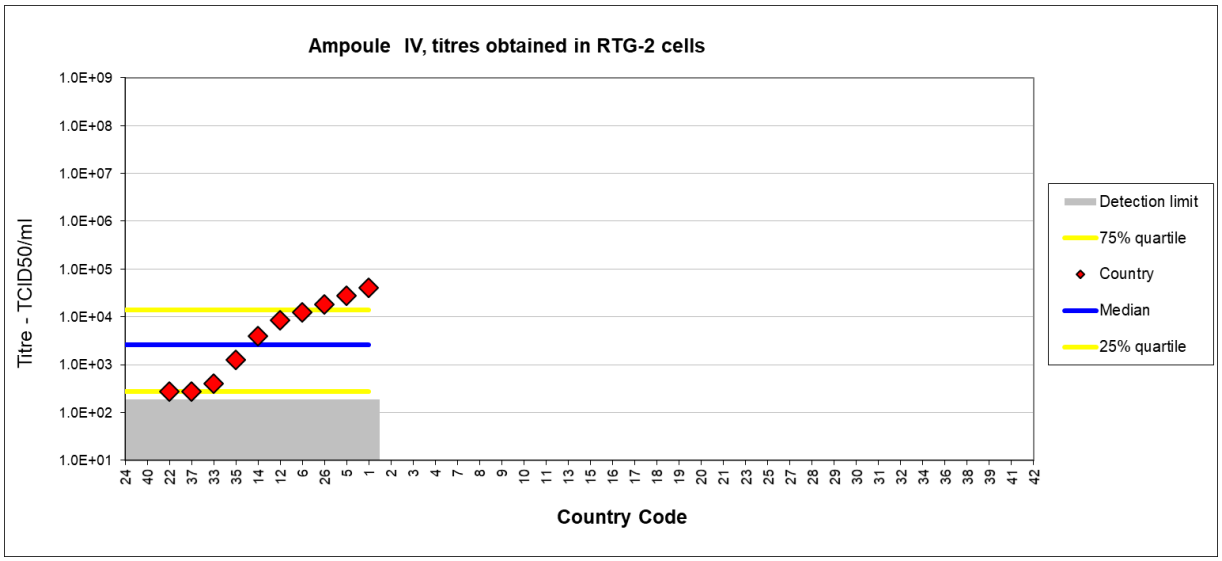


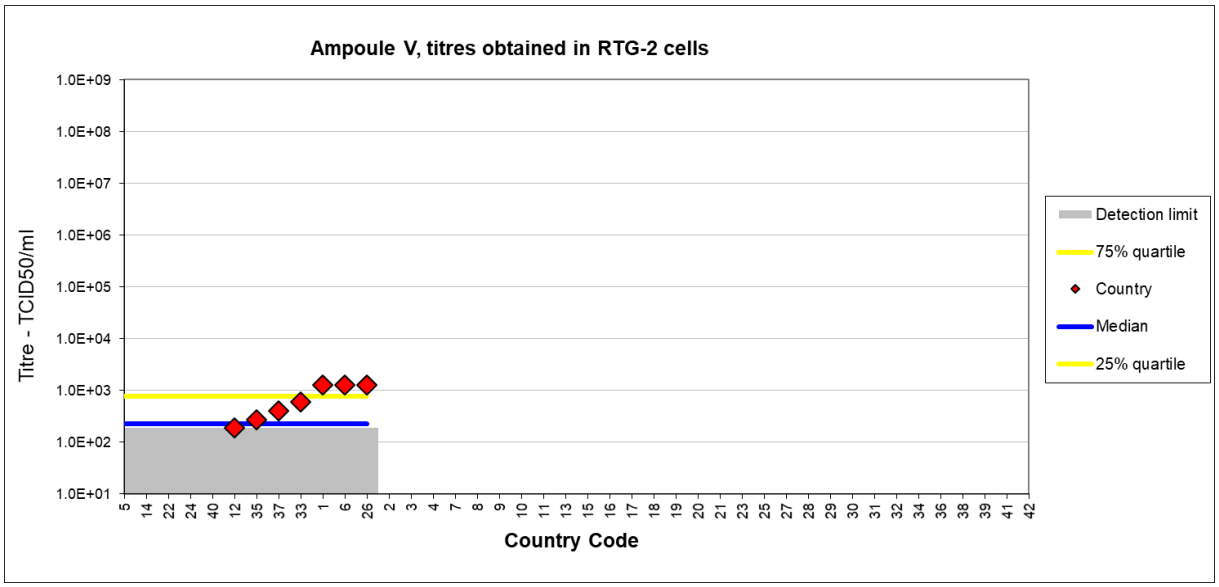


25% quartile (lower yellow line) are under the detection limit (grey shadow)



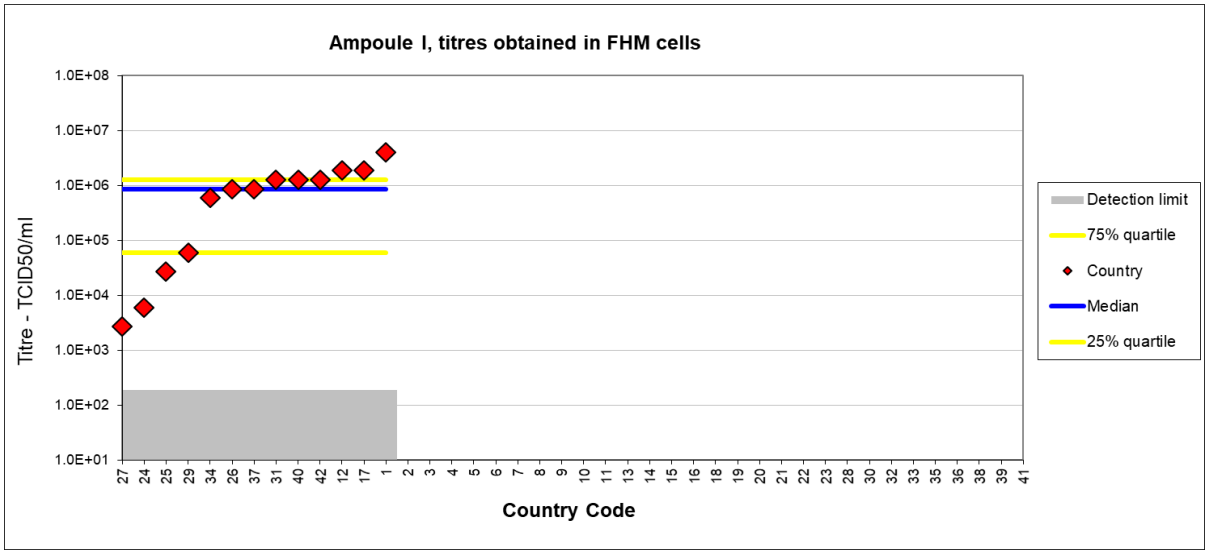
25% quartile (lower yellow line) are under the detection limit (grey shadow)

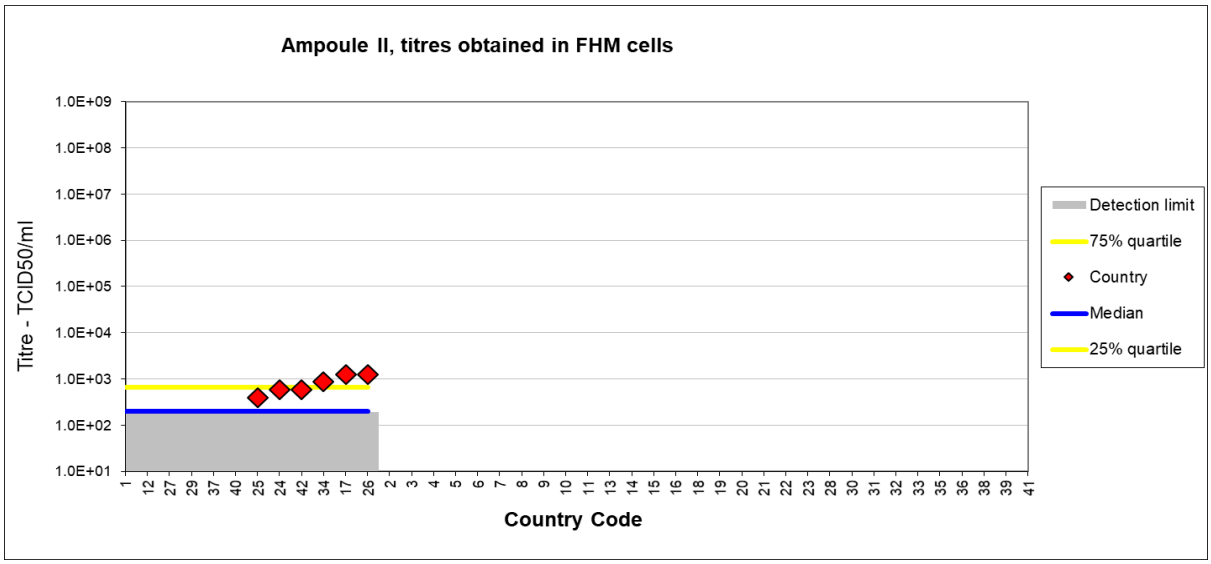




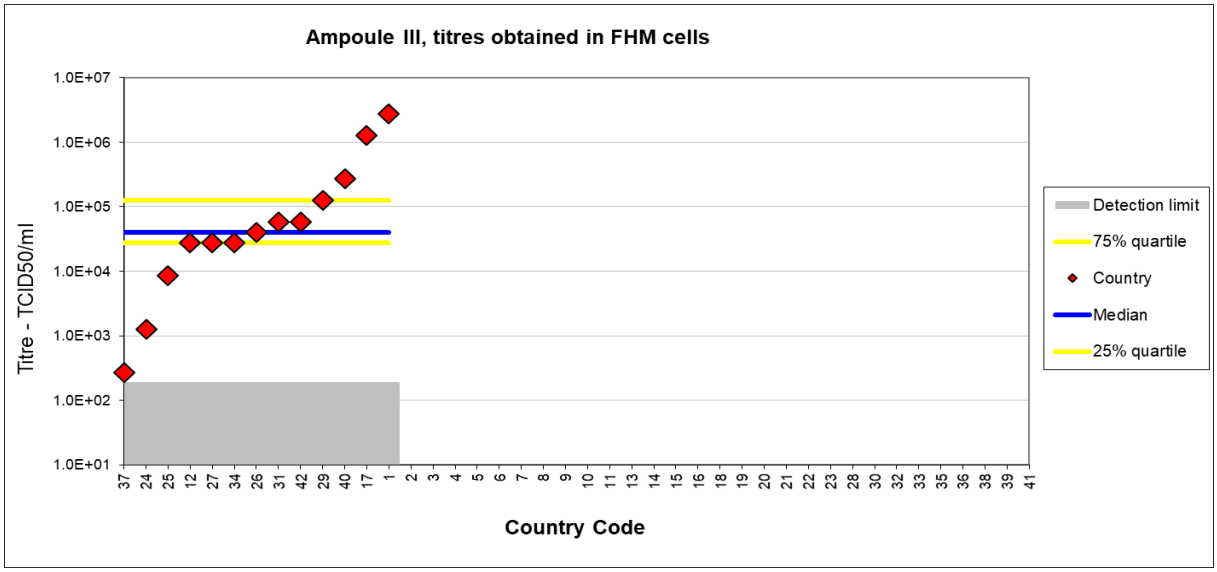
25% quartile (lower yellow line) are under the detection limit (grey shadow)

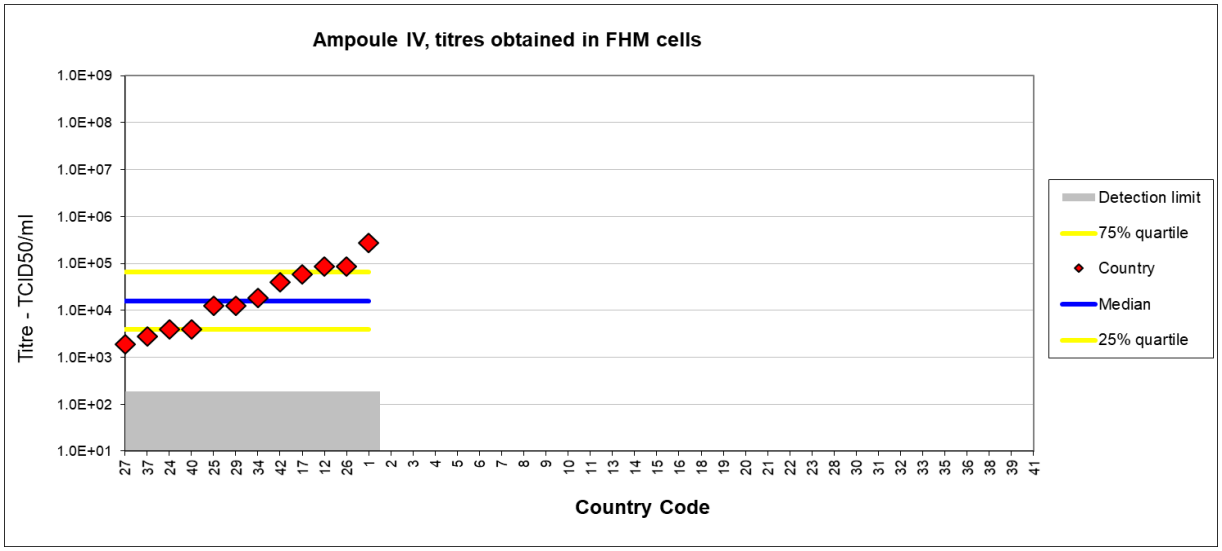
Figure 8. Virus titres obtained in FHM cells. For further details see description at Figure 5

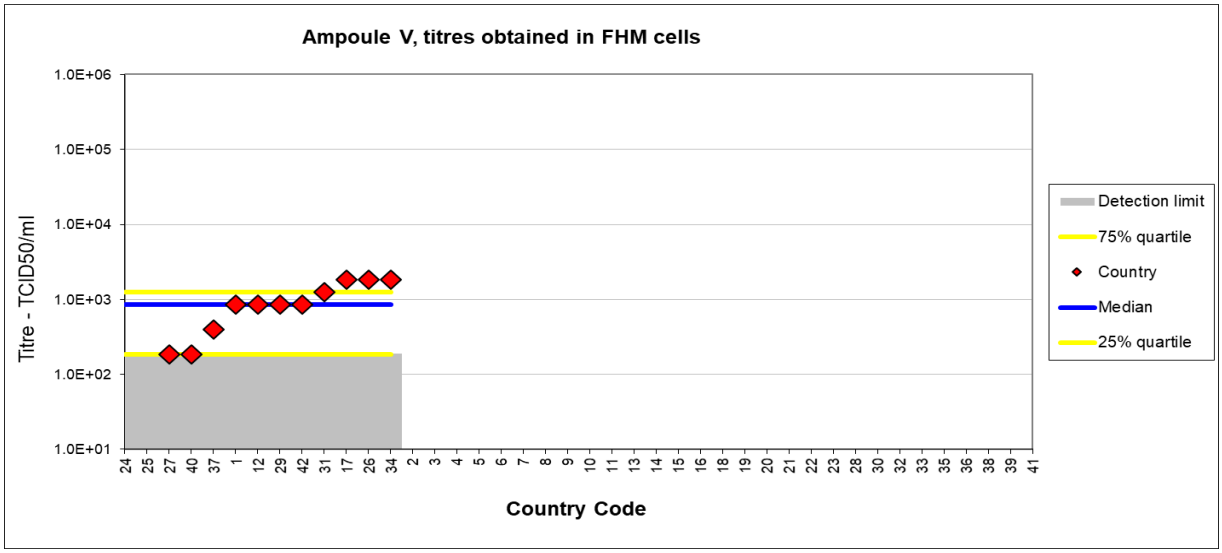




25% quartile (lower yellow line) are under the detection limit (grey shadow)




Ampoule IV, titres obtained in FHM cells
Titre - TCID50/ml
1.0E+09
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
27 37 24 40 25 29 34 42 17 12 26 1 2 3 4 5 6 7 8 9 10 11 13 14 15 16 18 19 20 21 22 23 28 30 31 32 35 36 38 39 41
Country Code
Detection limit
75% quartile
Country
Median
25% quartile


Ampoule V, titres obtained in FHM cells
Titre - TCID50/ml
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
24 25 27 40 37 1 12 29 42 31 17 26 34 2 3 4 5 6 7 8 9 10 11 13 14 15 16 18 19 20 21 22 23 28 30 32 35 36 38 39 41
Country Code
Detection limit
75% quartile
Country
Median
25% quartile

Identification of content

- 40 laboratories out of 42 participants analysed for all viruses; 30 of these laboratories correctly identified all viruses in all ampoules.
- Out of 42 participants one laboratory did not test for Ranavirus, one laboratory did not test for SVCV, both laboratories correctly identified all other viruses in the ampoules.

Ampoule I – IHNV (DK 21-4070-1)

- 42 laboratories correctly identified the isolate as IHNV in ampoule I

Ampoule II – ECV (562/92)

- 38 laboratories correctly identified the isolate as ECV or "Not EHNV" in ampoule II by sequencing or REA.
- 1 laboratory used a PCR which distinguishes EHNV from ECV by the size of the product in parallel to the traditional method (MCP amplification + sequencing).
- 1 laboratory identified Ranavirus but did not specify if the isolate was the listed EHNV or not by sequencing or REA.
- 1 laboratory did not find any virus in ampoule II
- 1 laboratory answered EHNV even though the blast show ECV
- 1 laboratory did not test for Ranavirus

Ampoule III – SVCV (DK-203273)

- 38 laboratories correctly identified the isolate as SVCV in ampoule III
- 2 laboratories correctly identified the SVCV but also found another virus not present in the ampoule (one laboratory additionally identified IHNV and one IPNV)
- 1 laboratory did not find any virus in ampoule III
- 1 laboratory do not test for SVCV

Ampoule IV – VHSV (NO-2007-50-385)

- 42 laboratories correctly identified the isolate as VHSV in ampoule IV

Ampoule V – IHNV (DK 21-4070-1)

- 37 laboratories correctly identified the isolate as IHNV in ampoule V
- 5 laboratories did not find any virus in the ampoule.

Scores

Starting with proficiency test 2003 we have provided a scoring system for the identification part of the proficiency tests. We have assigned a score of 2 for each correct answer/identification, giving the possibility for obtaining a maximum score of 10 (Table 3).

- Ampoule I: IHNV identification was given the score 2.
- Ampoule II: ECV or 'Not EHNV' identification was given the score 2.
  No identification of the Ranavirus by sequencing was given the score 1.
  No identification of the Ranavirus by sequencing or wrong interpretation was given the score 0.
- Ampoule III: SVCV identification was given the score 2
  SVCV identification and findings of other virus was given the score 1
- Ampoule IV: VHSV identification was given the score 2
- Ampoule V: IHNV identification was given the score 2.

In relation to the ranaviruses included in the ILPT, full score was given only in case one laboratory could isolate the virus and fully identify the isolate by means of sequencing.
Although it is acknowledged that, theoretically, other methods can be used to discriminate (e.g. specific qPCR assay) these have not been fully validated or the data of such validation are not available, hence we have considered that the result is not corroborated and fully supported from the diagnostic method used.

Out of 42 laboratories participating in the PT 1 2022, 30 obtained score 10/10.
The score 8/8 was assigned to two participant as they did not test for Ranavirus or SVCV.
12 laboratories scored below 100% due to wrong or no identification by sequencing of the Ranavirus or due to not finding the virus in the ampoule or also finding a virus not present in the ampoule.

Cells applied for solving the test

Within the panel of cell lines available in the legislation the following ones were used by the participants:

- 37 laboratories used BF-2 cells
- 36 laboratories used EPC cells
- 12 laboratories used RTG-2 cells
- 13 laboratories used FHM cells
- 7 laboratories used CHSE-214 cells
- 3 laboratories did not titrate and 1 laboratory did only use BF-2 cells

The combination of EPC and FHM cells or BF-2 and RTG 2 alone is not valid according to EURL diagnostic manuals [2] The laboratories are encouraged to include the use of BF-2 cells or RTG 2 cells and EPC cells or FHM cells.

The median titre calculated from the submitted results of each ampoule and in each cell line is illustrated in Figure 9.

It appears that:

- Ampoule I (IHNV DK 21-4070-1) replicates on all four cell lines however it grows a little less efficiently on BF-2 but within two log difference.
- Ampoule II (ECV 562/92) replicate all four cell lines however it grows poorly on RTG-2 and FHM.
- Ampoule III (SVCV DK-203273) replicates on all four cell lines however it grows less efficiently on RTG-2
- Ampoule IV (VHSV NO-2007-50-385) replicates on all four cell lines however it a little less efficiently on RTG-2.
- Ampoule V (IHNV DK 21-4070-1 in low titre) replicates on EPC, RTG-2 and FHM however it grow poorly on RTG-2 and dos not replicates on BF-2.

As from Table 4-8 the variations in titres between laboratories was high – with more than 7 log differences from the lowest to the most sensitive performances. Laboratories scoring low titres should definitely consider to exchange their cell lines with more sensitive ones or assess if the performance of their cells could be improved and the ones with a high titre should ensure to follow the correct titration procedure.

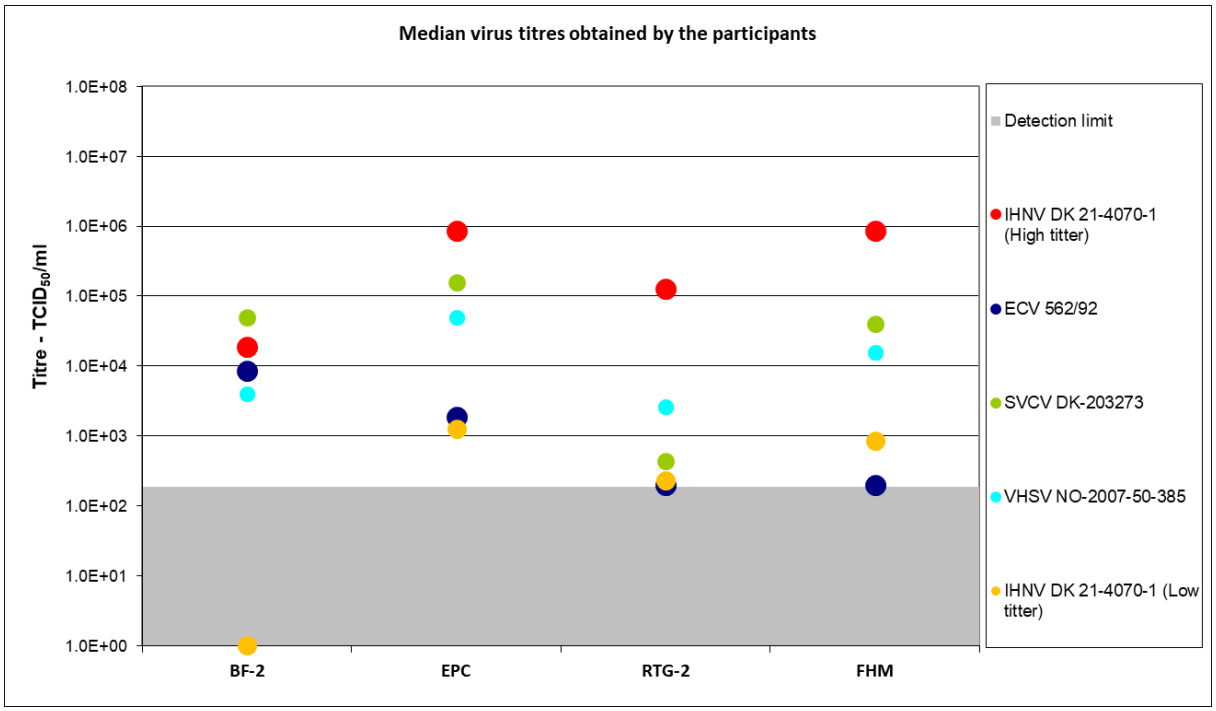


Figure 9. Median virus titres obtained by the participants in 4 different cell lines.

Ct. values comparison

We have encouraged participants to insert the Ct value in the spreadsheet if they have performed a real-time (RT-) PCR.
The Ct values obtained by the participating laboratories are summarised in tables 9 and figure 10

The Ct values cannot be directly compared due to the use of different methods, reagents and equipment for nucleic acid extraction and (RT)-qPCR. In order to align the results, participants has been asked to test the ampoules by molecular methods directly from the re-suspended material and not from the viral isolates, however it seems like a few participants have given the Ct values obtained from the viral isolates.
All Ct values submitted by the participants for each ampoule, are compared to each other. On these figures, the median values and the 25% and 75% inter-quartile range is displayed, the optimal value will be within these quartiles. A low Ct, below 25% quartile may be indicator of testing the isolate instead of the re-suspended material; conversely a very high Ct, beyond 75% quartile may indicate a lack of sensitivity in the method.

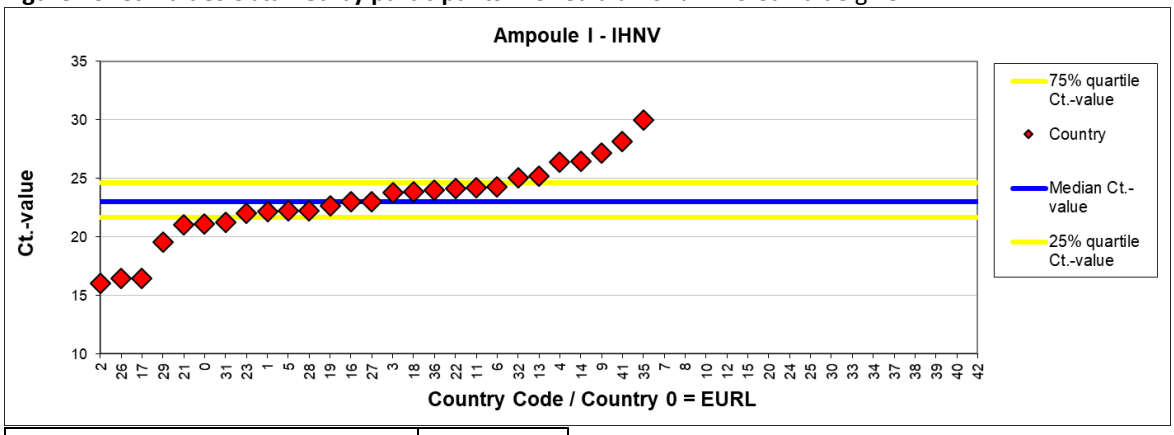
Table 9. Inter-Laboratory Proficiency Test, PT1, 2022 – Ct.-values.

Laboratory Code number	Ct. value Ampoule I (IHNV)	Ct. value Ampoule II (ECV)	Ct. value Ampoule II (SVCV)	Ct. value Ampoule III (VHSV)	Ct. value Ampoule IV (IHNV)
EURL	**21.1**	**28.9**	**22.2**	**24.7**	**31.5**
1	22.2	29.8	23.9	25.5	32.4
2	16.0	-	-	11.2	18.9
3	23.8	-	-	26.1	33.9
4	26.4	-	-	30.0	35.7
5	22.2	-	-	27.4	32.4
6	24.3	-	-	28.2	34.3
7	-	-	-	-	-
8	-	-	-	27.9	-
9	27.1	27.9	-	30.4	No Ct
10	-	-	-	-	-
11	24.2	-	-	26.5	33.8
12	-	-	-	-	-
13	25.2	-	-	24.8	39.2
14	26.4	-	26.3	30.2	36.2
15	-	27.9	-	-	-
16	23.0	-	-	24.9	32.2
17	16.5	-	-	15.6	16.7
18	23.8	-	-	25.2	34.4
19	22.6	-	22.9	27.6	32.5
20	-	-	23.6	-	-
21	21.0	-	24.9	32.4	33.5
22	24.1	-	-	29.6	-
23	22.0	-	-	26.7	-
24	-	-	-	34.2	-
25	-	-	-	-	-
26	16.4	-	-	24.5	25.7
27	23.0	26.7	-	27.6	32.2
28	22.2	-	-	26.3	32.2
29	19.6	26.9	30.0	30.6	30.3
30	-	-	-	-	-
31	21.3	25.4	24.7	33.8	32.2
32	25.0	-	-	27.0	35.0
33	-	-	-	30.0	-
34	-	-	-	31.8	-
35	30.0	26.4	23.9	26.2	39.8
36	24.0	-	24.6	31.2	36.1
37	-	-	-	-	-
38	-	-	-	-	-
39	-	-	-	-	-
40	-	-	-	-	-
41	28.1	-	-	25.4	23.1
42	-	-	-	30.1	-

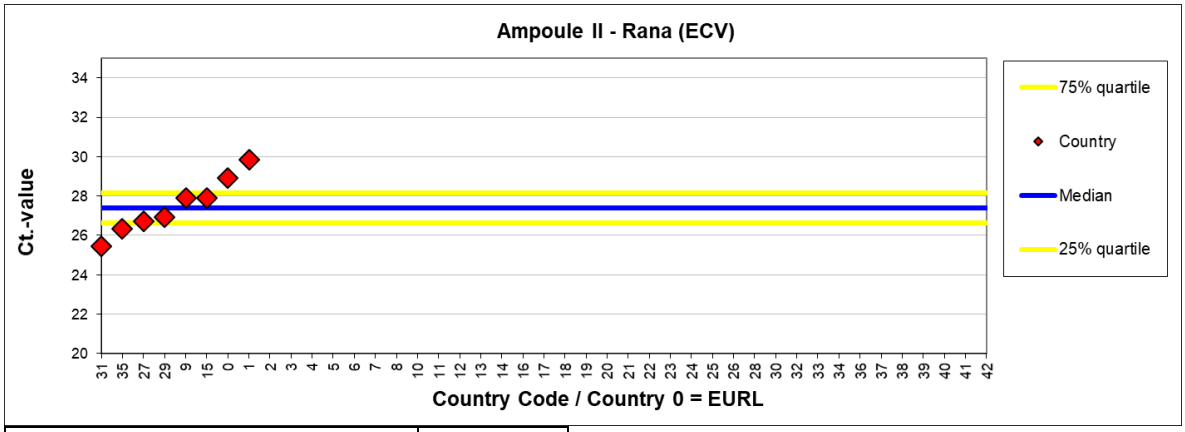
- No Ct-value given by the participating laboratory.

The Ct. values obtained from each participating laboratory are represented graphically in Figures 10. On these figures, the median Ct value and the 25% and 75% inter-quartile range is displayed.

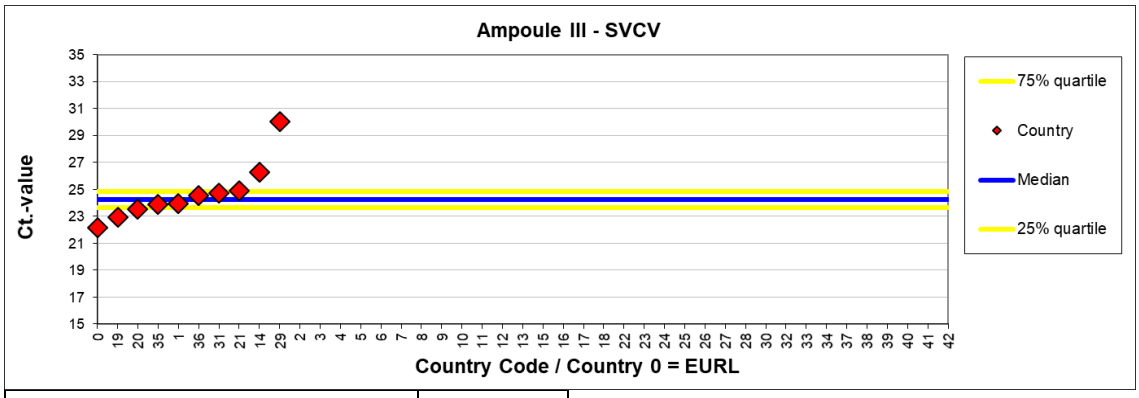
Figure 10. Ct. values obtained by participants. No red diamond = No Ct. value given



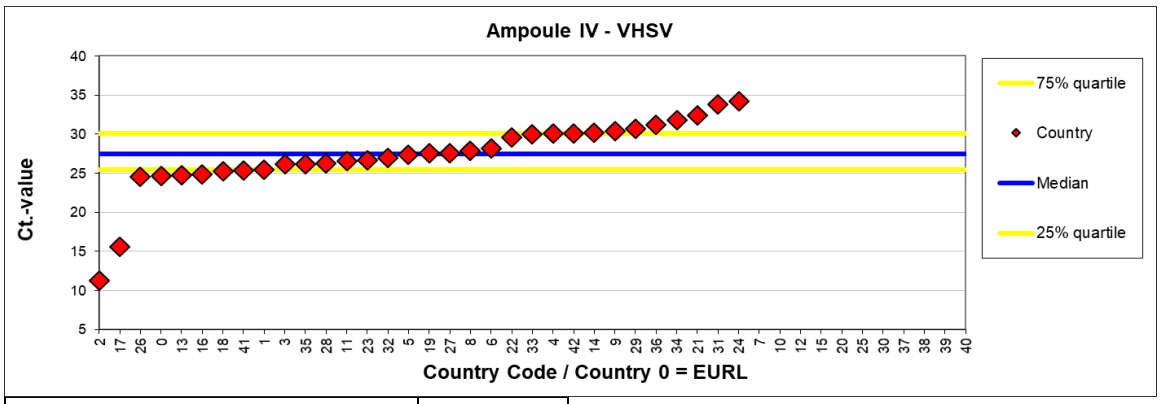
Number of laboratories	27
Median Ct.-value	23.0
Maximum Ct.-value	30.0
Minimum Ct.-value	16.0
25% quartile Ct.-value	21.6
75% quartile Ct.-value	24.6



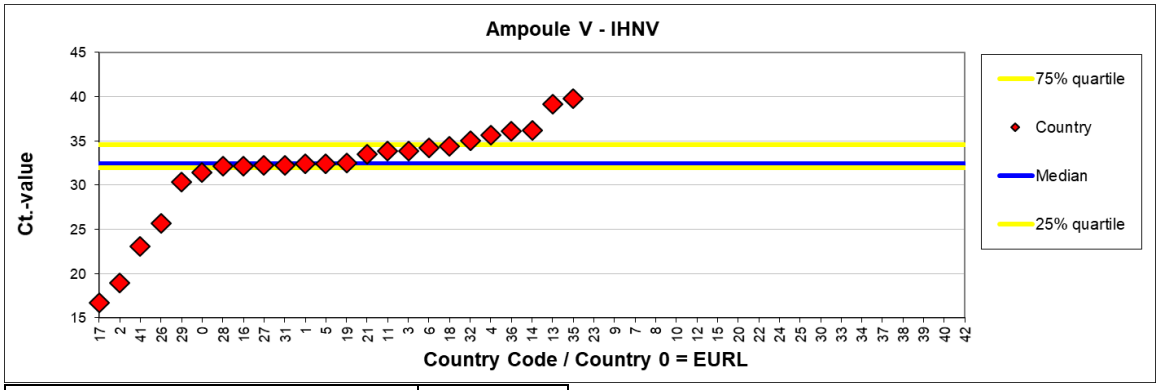
Number of laboratories	8
Median Ct.-value	27.4
Maximum Ct.-value	29.8
Minimum Ct.-value	25.4
25% quartile Ct.-value	26.6
75% quartile Ct.-value	28.1



Number of laboratories	10
Median Ct.-value	24.2
Maximum Ct.-value	30.0
Minimum Ct.-value	22.2
25% quartile Ct.-value	23.6
75% quartile Ct.-value	24.9



Number of laboratories	32
Median Ct.-value	27.5
Maximum Ct.-value	34.2
Minimum Ct.-value	11.2
25% quartile Ct.-value	25.5
75% quartile Ct.-value	30.1



Number of laboratories	26
Median Ct.-value	32.5
Maximum Ct.-value	39.8
Minimum Ct.-value	16.7
25% quartile Ct.-value	32.0
75% quartile Ct.-value	34.6

Genotyping and sequencing

We have encouraged participants to genotype the identified viruses though it has not been a mandatory task. As ranaviruses are included in the test, it is mandatory to do sequence analysis in order to discriminate EHNV from the non-listed ranaviruses. For IHNV and VHSV we still encouraged participants to genotype isolates. An overview of the genotyping results obtained for PT1 by all participants is displayed in the following table 10.

The EURL has disclosed the content of the ampoules after deadline for delivering results.

Table 10. The genotyping results obtained for PT1 by all 42 participants

Code number	Ampoule I	Ampoule II	Ampoule III	Ampoule IV	Ampoule V
	IHNV DK 21-4070-1 Genogroup E	**ECV 562/92**	**SVCV 56/70 Genotype Ia**	**VHSV NO-2007-50-385 Genotype IIIb**	**IHNV DK 21-4070-1 Genogroup E**
1	E	Not EHNV	Ia	III	E
2	0	ECV	0	0	0
3	Genotype = E	0	Genogroup = Id	Genogroup = III	Genotype = E
4	E	Not EHNV	0	III	E
5	E	ECV	Ia	III	E
6	E	Not EHNV	Ia	III	E
7	E	Not EHNV	0	0	0
8	IHNV	European Catfish Virus	SVCV	VHSV	0
9	E	Ranavirus-NOT EHNV	Genogroup 1a	III	E
10	M	Not EHNV	0	1b	M
11	E	NOT EHNV	Genogroup 1a	III	E
12	E	European catfish virus	Ia	type III	E
13	E	not EHNV	1a	III	0
14	0	0	0	0	0
15	E	Ranavirus - not EHNV	1a	III	E
16	E	Not EHNV	0	III	E
17	E	0	1a	III	E
18	E	Not EHNV	0	III	E
19	E	Not EHNV	0	III	E
20	E	Not EHNV	Genogroup 1(a)	III	E
21	E	Not EHNV	0	III	0
22	E	not EHNV	Ia	III	0
23	E	ECV (Ranavirus-Not EHNV)	0	III	E
24	E	European Catfish virus(Ranavirus)	Ia	III	-
25	E	Ranavirus	Ia	III	0
26	E	Not EHNV	Ia	III subtype III	E
27	E	Not EHNV	0	III	E
28	IHN Genotype: E	Not EHN	Genotype: Ia	Genotype: III	IHN Genotype: E
29	0	0	0	0	0

30	Genogroup M	Not EHNV	Genogroup a	Genogroup III	Genogroup M
31	IHN E	European sheatfish virus	SVCV Genogroup 1a	VHS genogroup 3	IHN E
32	M	NOT EHNV	1a	III	M
33	E	0	Ia	III	E
34	Genogroup M	0	Type Ia	0	Genogroup M
35	E	Not EHNV	1a	III	E
36	Genotype: E, Germany	epizootic haematopoietic necrosis virus	Gynotype: 1a	Genotype: Ia; Norway	Genotype: E, Germany
37	0	Not EHNV	0	0	0
38	0	0	0	0	0
39	0	0	0	0	0
40	E	Not EHNV	Ia	III	E
41	Genogroup E	ECV	Genotype Ia	Genotype III	Genogroup E
42	E	Not EHNV	1a	III	E

[1] This laboratory doesn't test for ranavirus
[2] This laboratory doesn't test for SVCV
[3] This laboratory has not provided corroborating data to support the finding of EHNV in ampoule I and Ranavirus in ampoule III

76% of the participating laboratories obtained 100% success rate in PT1.

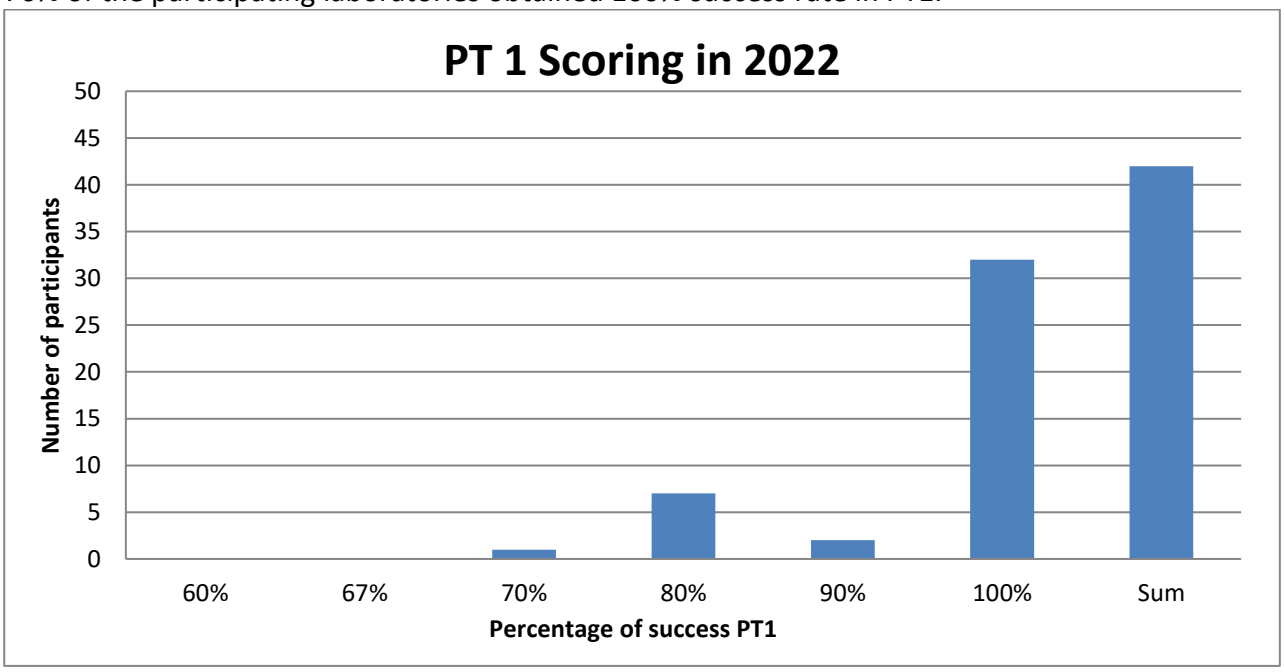


Figure 11 Success-rate of participating laboratories 2022 for PT1

Résumé and concluding remarks PT1

90% of the parcels were delivered by the shipping companies within two week and 100% was delivered within 26 days.
Overall 32 out of 42 participants scored 100% success rate; out of the 10 laboratories which underperformed only one participant scored <100% for the sole reason that they did not back up their concluding results of ampoule II (ECV) with sequencing. 5 laboratories did not find the virus in ampoule V (low titre IHNV). 2 laboratories had a contamination in ampoule II (SVCV) and one laboratory answered EHNV in ampoule II even though the sequence show ECV. Suggestions to improve on underperformance will be provided individually to each laboratory.

In this report (Figures 5-8), all the viral titres submitted by participants are compared to each other. In this way, the titres obtained by each laboratory are plotted in relation to the combined submitted data set and each participating laboratory is able to compare the sensitivity of its cell lines to the sensitivity of those used by the other participants. We recommend all participants to evaluate the sensitivity of the cells used in their laboratory in relation to the diagnostic purpose, especially as it appears that the variations in titres between laboratories was rather high – with more than 7 log differences from the lowest to the most sensitive performances. Laboratories scoring low titres should definitely consider to exchange their cell lines with more sensitive strains or assess if the performance of their cells could be improved and the laboratories scoring very high titres should ensure that the titration procedure is properly implemented.

Although the direct comparison of Ct Values cannot be done due to specific differences in laboratory, reagents, assay setup etc. the table included in this report may provide valuable information for the participating laboratories, in assessing their results with other laboratories as well as with the EURL, and evaluate the working pipeline in the molecular laboratory, in case of significant differences in the results are obtained. Further specifications both on the assay set up and on the working pipeline will be provided at the specific meeting in April.

Concerning sequence analysis this report can act as tool so that each laboratory can compare its own sequence analysis and genotyping.
The sequencing and genotyping of VHSV and IHNV is well implemented in the network of laboratory participating in this Inter-Laboratory proficiency test , 34 laboratories have sequenced VHSV, and 31 have correctly genotyped the isolate in ampoule IV as Genotype III. 36 laboratories have sequenced IHNV in ampoule I, and 35 have correctly genotyped the IHNV as Genogroup E or M (four M)
Since genogroup “E”, is being one of the first isolate discovered in Europe, it likely to belong to M Genogroup, hence “M” also is been considered correct answer.

The EURL provides the annual proficiency test, collates the data and process the figures so that individual laboratories can see how they perform in relation to the other participants. We will also take the opportunity to provide a comment to participants regarding submitted results if relevant. Furthermore we encourage all participants to contacts us with any questions concerning the test or any other diagnostic matters.

The results presented in this report will be further presented and discussed at the 27th Annual Workshop of National Reference Laboratories for Fish Diseases to be held 30th and 31st of May , 2023.

Proficiency test 2, PT2

Four ampoules containing lyophilised cell culture supernatant were delivered to the same laboratories that participated in PT1 with the exception of one laboratory that participated only in PT1.

Content of ampoules

The viruses were propagated on each of their preferred cell line and the supernatants were collected and filtrated through a 45 µm filter, mixed with equal volumes of 2% w/v lactalbumin hydrolysate solution and lyophilized in glass ampoules.
Before the ampoules were prepared, the concentration of the viral stocks was analysed by the KHV real-time PCR protocol described by Gilad et *al*. (2004) [8], the SAV real-time RT-PCR protocol described by Hodneland et al. (2006) [10], and the ISAV real-time RT-PCR protocol described by Snow et *al*. (2006) [12] .
Each viral stock was further identified by PCR and sequencing. For KHV according to the method described by Bercovier et *al*. (2005) [9] , for SAV according to the conventional PCR targeting segment E2 described by Fringuelli et al. (2008) [11] and for ISAV with conventional RT-PCR protocol described by Mjaaland et *al*. (2002) [13].
The details of the virus isolates used in the proficiency test 2 are outlined in table 11.

Table 11. Content in each ampoule with reference to culture conditions and major publications of the included pathogens.

Code	Specifications/References
Ampoule VI: BLANK	**Cell supernatant from BF-2 cells 02/20** **Passage No.:** 35. **Pass.** 15/3-22**.**
Ampoule VII: CyHV-3/KHV	**Koi Herpesvirus isolate KHV 1287** **Received from**: Dr. Kei Yuasa, National Research Institute of Aquaculture, Japan Isolate from Common Carp (Cyprinus Carpio), from river in the Okayama region, Japan in 2012.
Ampoule VIII: ISAV	**Infectious Salmon Anaemia Virus. ISAV 2016-70-1297_Vir4415** ISAV HPRΔ isolate from Atlantic salmon in Norway in 2016. **Received from** Norwegian Veterinary Institute. **Genbank accession number** MK216307

Code	Specifications/References
Ampoule IX: SAV	**SAV 6 – PD** SAV VI: Pancreas Disease Virus, Ireland F104596 **GenBank accession numbers:** EF675499 (nsp3 gene); EF675547 (E2 gene) **Reference on isolate:** Phylogenetic analyses and molecular epidemiology of European salmonid alphaviruses (SAV) based on partial E2and nsP3 gene nucleotide sequences. E Fringuelli, H M Rowley, J C Wilson, R Hunter, H Rodger and D A Graham Journal of Fish Diseases 2008, 31, 811–823 doi:10.1111/j.1365-2761.2008.00944.x

Testing of the PT2 test

The PT2 test was prepared and tested according to protocols accredited under DS/EN ISO/IEC 17043. Prior to the distribution we tested 5 ampoules of each virus preparation, by real-time PCR (Gilad et *al*. (2004))[8] for KHV, by real-time RT-PCR (Snow et *al*. (2006)) [12]for ISAV and by real-time RT PCR (Hodneland et al. (2006))[10] for SAV, to ascertain identity and homogeneity of the content in the ampoules (Figure 12). As a result all the standard deviations were below 1 Ct. value. Furthermore, after deadline for handling in results and minimum 3 months after lyophilisation and storage in the dark at 4°C, the content of the ampoules were tested to assess their stability (Table 12 and Figure 13). Conventional PCR/RT-PCR fragments were sequenced and so was the HPR region in segment 6 of the ISAV isolates.

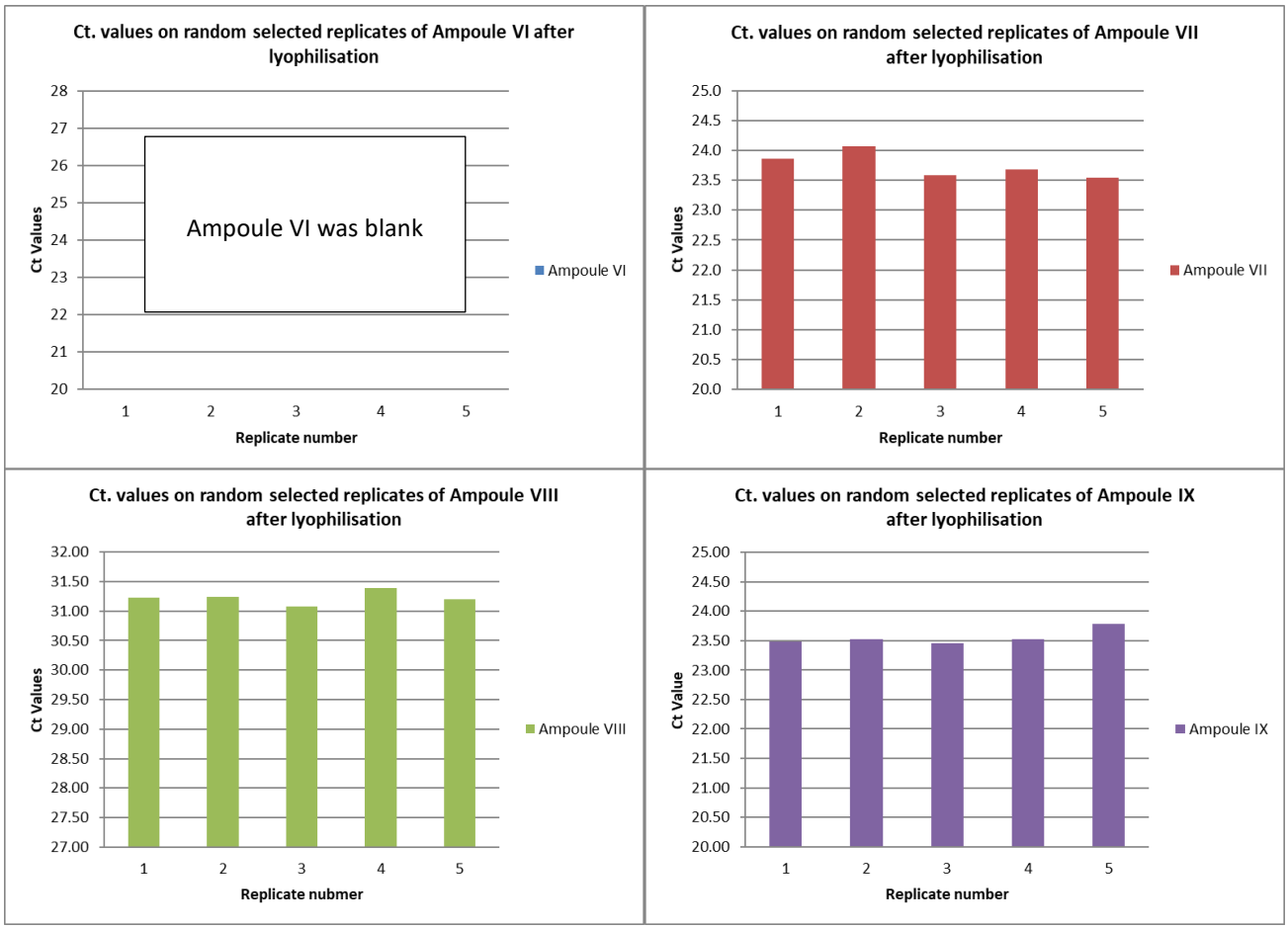


Figure 12, Ampule VI (Blank), VII (KHV), VIII (ISAV), IX (SAV) tested shortly after lyophilisation to assess homogeneity of the content.

Table 12: Ct-value of ampoules VI to IX tested before and immediately after lyophilisation and after deadline for handling in results**.**

Ampoule	Content	Cell line	EURL before lyophilization	EURL right after lyophilization	EURL after deadline for answering
Ampoule VI	**Blank**	a	No Ct	No Ct	No Ct.
		b		No Ct	
		c		No Ct	
		d		No Ct	
		e		No Ct	
			No Ct	**No Ct**	**No Ct**
Ampoule VII	**KHV**	a	21.88	23.87	23.64
		b		24.07	
		c		23.58	
		d		23.68	
		e		23.54	
			21.88	**23.75**	**23.64**
Ampoule VIII	**ISAV**	a	26.83	31.23	31.12
		b		31.24	
		c		31.08	
		d		31.39	
		e		31.20	
			26.83	**31.23**	**31.12**
Ampoule IX	**SAV**	a	19.3	23.49	23.41
		b		23.52	
		c		23.45	
		d		23.52	
		e		23.78	
			19.30	**23.55**	**23.41**

The lyophilisation procedure caused a significant virus reduction in all four ampoules (between 2-5 Ct. values) as detected by real-time PCR or real-time RT-PCR.
For each ampoule no other pathogens than the expected were detected.

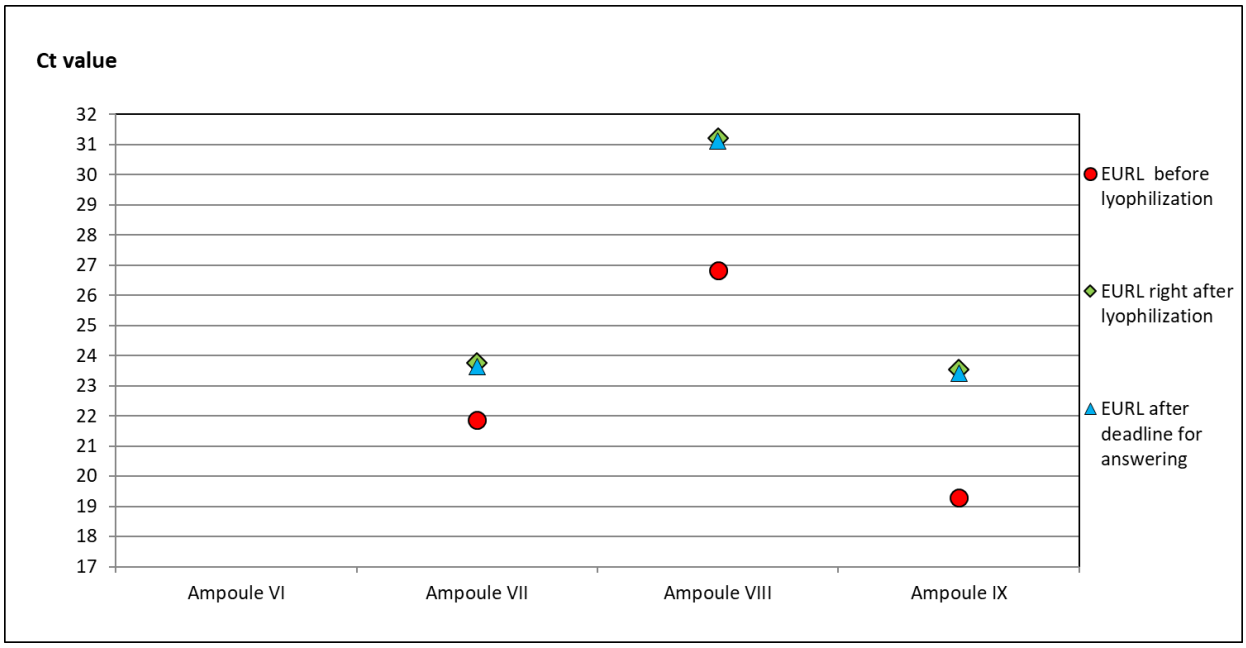


Figure 13, Ampoule VI, VII, VIII and IX tested before and after lyophilisation and after deadline for handling in results.

The lyophilisation procedure caused a significant virus reduction in all four ampoules (between 2-5 Ct. values) as detected by real-time PCR or real-time RT-PCR.
For each ampoule no other pathogens than the expected were detected.

Pathogen identification

In PT2, participants were asked to identify any of the fish viruses ISAV and KHV according to diagnostic procedures described in the EURL diagnostic manuals [2]. Bearing in mind that the test ampoules also could contain other viruses. Participants were expected to use their normal PCR or real-time PCR methods for detection of KHV and their normal RT-PCR or real-time RT-PCR methods for detection of ISAV.
It was not mandatory to grow KHV and ISAV on cell cultures in this test, but the viruses had not been inactivated, so, theoretically, the isolates should be viable.
Also this year, the panel of pathogens to be investigated included SAV – salmonid alpha virus. Since this is not a listed disease in the European legislation the participation was voluntary and therefore the participants were asked to declare if the ampoules were tested for SAV or not.
In order to obtain uniform answers, participants were requested to download a spreadsheet available from the https://www.eurl-fish-crustacean.eu, insert results in this and return by email.
The results from participating laboratories are shown in table 13.

Table 13. Inter-Laboratory Proficiency Test, PT2, 2022 - Virus identification.

Laboratory code number	Score	Ampoule VI	Ampoule VII	Ampoule VIII	Ampoule IX
		BF-2 cells	KHV, (CyHV-3)	HPRΔ ISAV	SAV6
1	8/8	Not ISAV, not SAV, not KHV	KHV	HPR deleted ISAV	SAV
2	6/6	no KHV; no ISAV	KHV	HPR-deleted ISAV	no KHV; no ISAV
3	8/8	Negative	KHV	ISAV-HPR deleted	SAV
4	8/8	No virus	KHV	HPR-deleted ISAV	SAV
5	8/8	no ISAV, no KHV, no SAV	KHV Japanese lineage	HPR-deleted ISAV	SAV
6	8/8	negative	KHV	HPR-deleted ISAV	SAV
7	5/6	NO KHV NO ISAV	KHV	ISAV	NO KHV NO ISAV
8	6/6	0	CyHV-3	ISAV	0
9	8/8	Not ISAV, Not SAV, Not KHV	KHV	HPR-deleted ISAV	SAV
10	8/8	Blank	KHV	HPR-deleted ISAV	SAV
11	8/8	NEG	KHV	HPR-deleted ISAV	SAV
12	8/8	None	KHV	ISAV	SAV
13	6/6	0	KHV	HPR-deleted ISAV	0
14	7/8	Negative	KHV	ISAV	SAV
15	8/8	N/A	KHV	HPR-deleted ISAV	SAV
16	8/8	No virus detected	KHV	HPR-deleted ISAV	SAV
17	8/8	-	KHV	HPR-deleted ISAV	SAV
18	6/6	Not ISAV,not KHV	KHV	HPR-deleted ISAV	Not ISAV,not KHV
19	8/8	Negative	KHV	HPR-deleted ISAV	SAV
20	8/8	NOT ISAV, KHV, SAV	KHV	HPR-deleted ISAV	SAV
21	8/8	Negative	KHV	HPR deleted ISAV	SAV
22	8/8	-	KHV	ISAV	SAV
23	7/8	Negative/Not detected	KHV	ISAV	SAV
24	8/8	-	KHV	ISAV	SAV
25	8/8	-	KHV	ISAV	SAV
26	8/8	NEG.	KHV	HPR-deleted ISAV	SAV
27	8/8	0	KHV	HPR-deleted ISAV	SAV
28	8/8	NO KHV, ISAV, SAV	KHV	ISAV	SAV
29	8/8	negative	KHV	HPR-deleted ISAV	SAV
30	8/8	Negative	KHV	HPR deleted ISAV	SAV
31	8/8	Negative	CyHV3 - KHV	HPR-deleted ISAV	SAV 6
32	8/8	-	KHV	HPR-deleted ISAV	SAV
33	8/8	Not ISAV, KHV, or SAV	KHV	HPR-deleted ISAV	SAV
34	8/8	Blank	KHV	ISAV	SAV
35	8/8	Negative	KHV	HPR-deleted ISAV	SAV
36	8/8	no virus detected	KHV	ISAV	SAV
37	8/8	No KHV No ISA No SAV	KHV	HPR-deleted ISAV	SAV
38		0	0	0	0
39	7/8	no virus	KHV	ISAV	SAV
40	8/8	0	KHV	HPR-deleted ISAV	SAV
41	8/8	not KHV, not ISAV nor SAV	KHV	ISAV	SAV
42	8/8	negative	KHV	ISAV	SAV

[1)] Did not test for SAV, [2)] Did not participate in PT2

All laboratories are asked to sequence the HPR region of ISAV isolates to distinguish from the pathogenic HPRΔ variant from ISAV HPR0 .

It was requested that the pathogens in the samples were not forwarded to third parts without having contacted the EURL for permission in advance.

Identification of content

- 41 laboratories submitted results
- 33 laboratories correctly identified all four ampoules (Blank, KHV, ISAV, SAV)
- All 41 laboratories tested for the two listed pathogens (KHV, ISAV)
- 36 laboratories tested for SAV
- 1 laboratory that did participate in PT 1 did not participate in PT2

Ampoule VI – Blank

- All 41 laboratories ruled out the presence of pathogens they were testing for, the answers varied from 'Not KHV, Not ISAV, Not SAV' to leaving the field empty.

Ampoule VII – KHV

- All 41 laboratories correctly identified KHV

Ampoule VIII – ISAV

- All 41 laboratories correctly identified ISAV hereof three laboratories did not sequenced and one laboratory has given the wrong variant.

Ampoule IX – SAV

- 36 laboratories correctly identified SAV
- 5 laboratories did not participate for SAV and answered '0' or 'no ISAV; no KHV'

Scores

We have assigned a score of 2 points for each ampoule (Table 13), giving the possibility for obtaining a maximum score of 8. Identifying the correct pathogen gives score of 2 points.
For the ISAV isolate in ampoule VIII, full score was given if ISAV virus was detected by molecular methods, and if the isolate was sequenced to discriminate between listed HPRΔ ISAV and non listed HPR0 ISAV.
Of the 41 laboratories submitting results 37 laboratories obtained maximum score. The maximum score was calculated according to the number of pathogen tested by the laboratory.
A laboratory could obtain a maximum score of 8 if tested for all three pathogens included (ISAV, KHV and SAV). A maximum score of 6 is given if only tested for ISAV and KHV.
4 laboratories scored below 100% due to wrong or no identification by sequencing of ISAV in ampoule VIII.

Ct. values comparison

We have encouraged participants to insert the Ct value in the spreadsheet if they have performed a real-time (RT-) PCR.

The Ct. values obtained by the participating laboratories are summarised in tables 14. The Ct. values obtained from each participating laboratory are represented graphically in Figures 14. On these figures, the median Ct value and the 25% and 75% inter-quartile range is displayed. Exceeding the values defined by the quartiles could suggest the laboratories to assess the laboratory procedures or the assay in use. A very low Ct below the 25% quartile may indicate that the sample tested derives from cell culture isolate. A very high Ct, beyond 75% quartile, may indicate that the assay in use or the procedure reduce the sensitivity of the method.

The Ct-values cannot be directly compared due to the use of different methods, reagents and equipment nucleic acid extraction and (RT)-qPCR.

Table 14. Inter-Laboratory Proficiency Test, PT2, 2022 – Ct.-values.

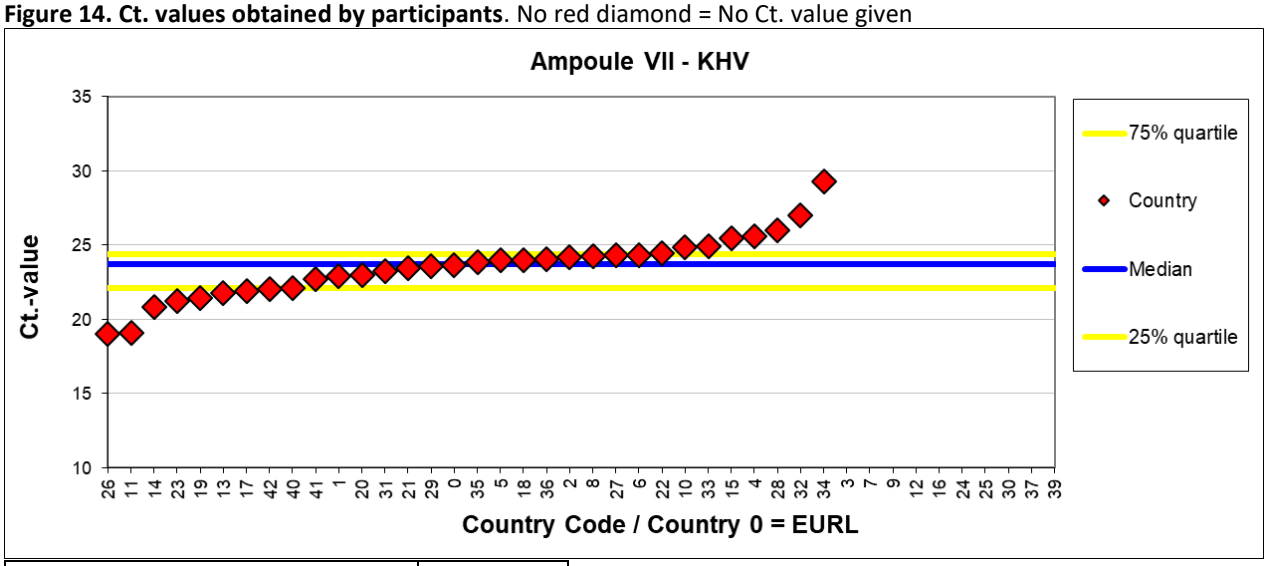
Laboratory Code number	Ct. value Ampoule VII (KHV)	Ct. value Ampoule VIII (ISAV)	Ct. value Ampoule IX (SAV)
EURL	23.6	31.1	23.4
1	22.9	31.68	23.79
2	24.2	36.00	-
3	-	30.78	26.58
4	25.6	31.51	27.64
5	24.0	-	31.60
6	24.3	34.69	30.71
7	-	-	-
8	24.3	32.48	-
9	-	39.77	26.93
10	24.9	35.90	27.76
11	19.1	35.72	29.77
12	-	-	-
13	21.8	27.72	-
14	20.8	32.50	34.93
15	25.5	38.93	-
16	-	30.40	30.90
17	21.9	35.86	-
18	24.0	31.07	-
19	21.4	-	-
20	23.0	33.26	27.92
21	23.4	-	-
22	24.5	35.36	-
23	21.2	-	-
24	-	-	-
25	-	-	-
26	19.0	23.80	-
27	24.3	32.90	28.80
28	26.0	37.46	-
29	23.6	34.00	28.01
30	-	-	-
31	23.2	33.77	27.86
32	27.0	32.00	25.00
33	24.9	31.85	-
34	29.3	34.19	31.00
35	23.8	31.91	28.98
36	24.0	32.31	28.93
37	-	34.12	24.85
38	-	-	-
39	-	32.50	29.80
40	22.1	32.31	25.67
41	22.7	-	-
42	22.0	30.28	-

- No Ct-value given by the participating laboratory.

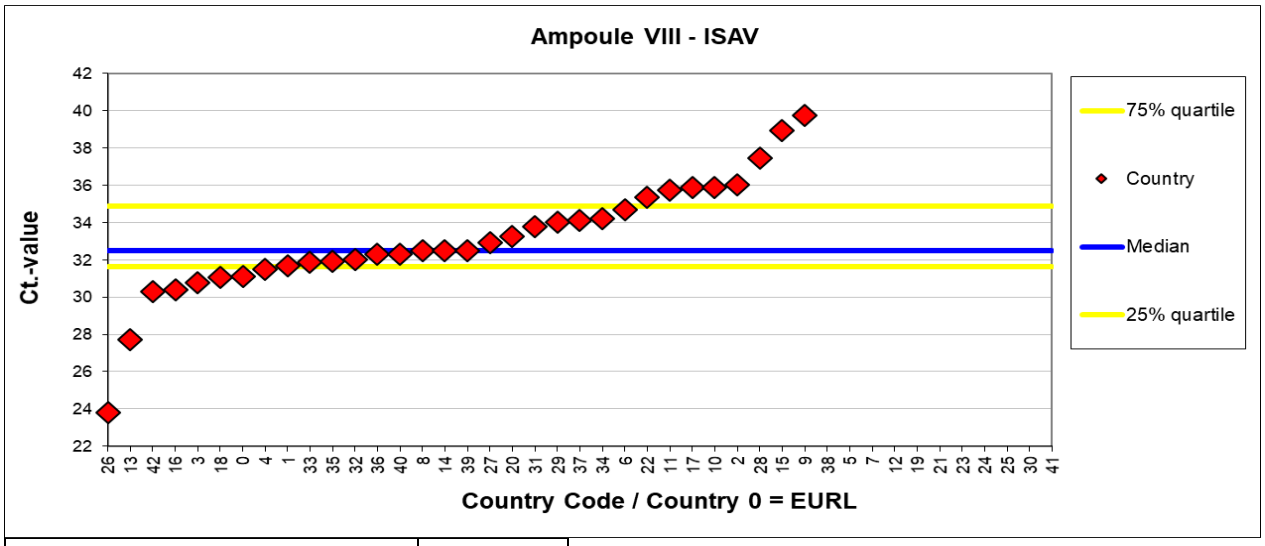
[1)] Did not participate in PT2.

Amp.VI was blank (and found blank by all participants) and thereby not included

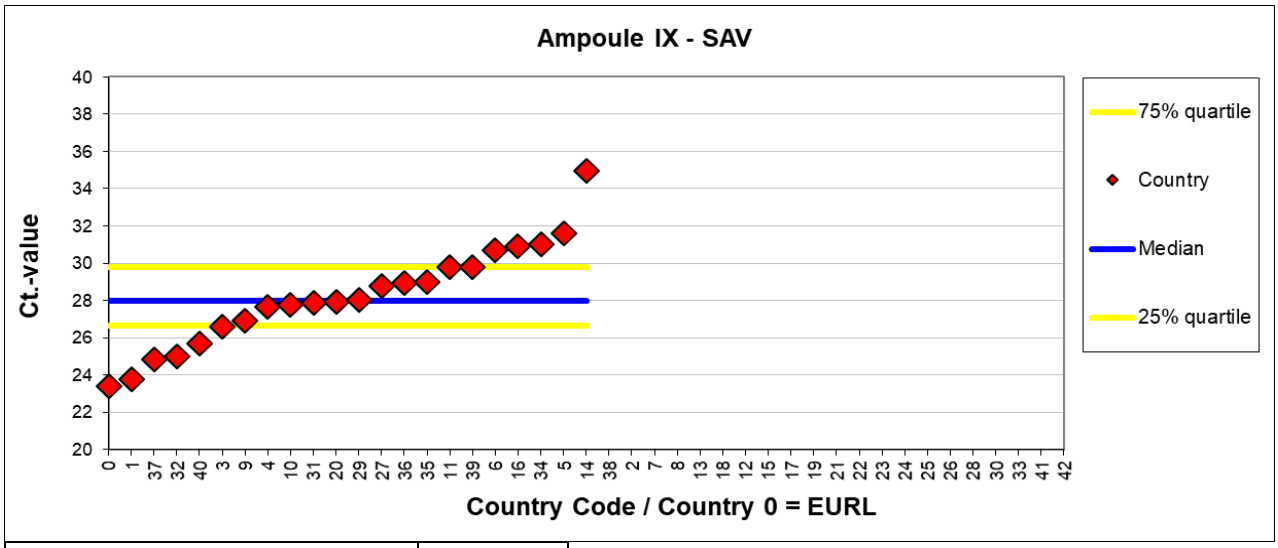
Figure 14. Ct. values obtained by participants. No red diamond = No Ct. value given



Number of laboratories	32
Median Ct.-value	23.7
Maximum Ct.-value	29.3
Minimum Ct.-value	19.0
25% quartile Ct.-value	22.1
75% quartile Ct.-value	24.4



Number of laboratories	32
Median Ct.-value	32.5
Maximum Ct.-value	39.8
Minimum Ct.-value	23.8
25% quartile Ct.-value	31.6
75% quartile Ct.-value	34.9



Number of laboratories	22
Median Ct.-value	28.0
Maximum Ct.-value	34.9
Minimum Ct.-value	23.4
25% quartile Ct.-value	26.7
75% quartile Ct.-value	29.8

Genotyping and sequencing

Participants were asked to sequence the HPR region of possible ISAV isolates and determine whether isolates included in the ampoules were HPRΔ ISAV currently listed in EU legislation or non-listed HPR0 ISAV, the correct characterization of HPRΔ ISAV has been calculated in the general score. Three laboratories did not sequence the ISAV isolate in ampoule VIII; one laboratory that sequenced the HPR segment wrongly concluded that the isolate was HPR0 ISAV. The identification of KHV didn't pose particular issues. Finally, regarding sequencing of SAV isolate in ampoule IX, only 1 out 29 of laboratories which performed genotyping of SAV, assigned the incorrect genotype and one sequenced but did not give any genotype.

An Overview of the genotyping results obtained for PT2 by all participants is displayed in the following table 15.

Table 15 The genotyping results obtained for PT2 by all 41 participants

Code number	Ampoule VII	Ampoule VIII	Ampoule IX
	KHV, CyHV-3	**HPRΔ ISAV**	**SAV6**
1	CyHV-3	HPR-deleted	6
2	0	HPR9	0
3	0	Genotype = G3	Subtype = SAV6
4	CyHV-3	HPR-deleted	0
5	CyHV3 JP lineage	ISAV HPR-deleted	VI
6	no genotypes but lineages	HPR-deleted	6
7	0	0	0
8	Cyprinid Herpesvirus 3	ISAV	0
9	CyHV3	HPR-deleted	6
10	CyHV (3)	HPR-deleted	1
11	CyHV-3	HRP-deleted	6
12	CyHV-3	pathogenic HPR-deleted ISAV (HPR9)	SAV6
13	CyHV3	HPR9	0
14	0	0	0
15	CyHV3	HPR-deleted	6
16	CyHV3	HPR-deleted	0
17	0	HPR-deleted	SAV 6
18	CyHV (3)	HPR-deleted	0
19	CyHV 3	HPR- deleted	VI
20	CyHV3	HPR-deleted	0
21	CyHV-3	HPR-deleted	6

22	CyHV - 3	HPR-deleted	6
23	CyHV3	HPR0	SAV6
24	CyHV-3	ISAV HPR-deleted, PR1	SAV6
25	CyHV-3	HPRdelta	SAV6
26	CyHV 3	HPR-deleted	6
27	0	HPR-deleted	6
28	CyHV3	ISAV_HPR-deleted	SAV6
29	0	HPR-deleted	0
30	CyHV-3	HPR-Deleted	SAV 6
31	CyHV 3	ISAV HPR deleted	SAV subtype 6
32	CyHV 3	HPR-deleted	6
33	CyHV3 - Asian	European- deleted	SAV6
34	0	HPR1, HPR deleted	0
35	CyHV3	HPR-deleted	6
36	CyHV-3; European E, CROATIA	ISAV: Norway HPR6	SPDV subtype: SAV6, Ireland
37	CyHV3	HPR-deleted	0
38	0	0	0
39	0	0	0
40	CyHV-3	HPR-deleted	6
41	CyHV-3	HPR deleted	Subtype 6
42	CyHV 3	HPR-deleted	6

[1)] Did not participate in PT2.

90% of the participating laboratories obtained 100% success rate in PT2.

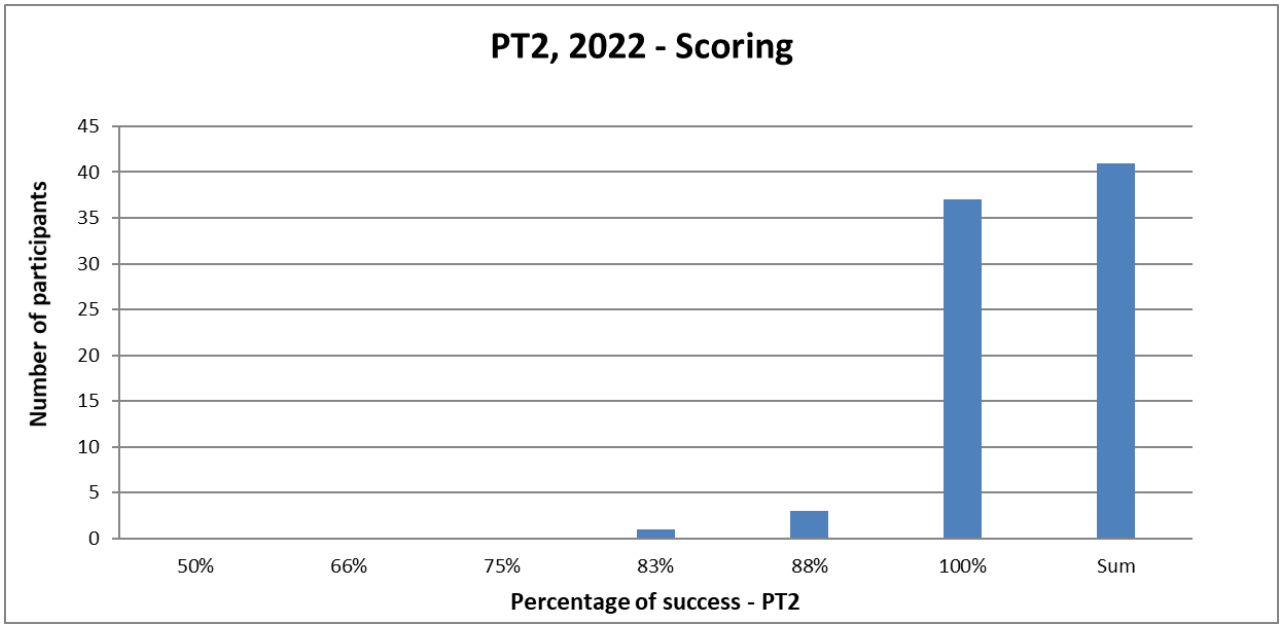


Figure 15 Success-rate of participating laboratories 2022 for PT2

Concluding remarks PT2

41 laboratories participated in PT2, 37 obtained 100% success rate. Out of the 4 laboratories which underperformed, three obtained a lower score because did not provide sequencing for the ISAV isolate in ampoule VIII. This point will be addressed directly with the participants that has underperformed.

All 41 laboratories correctly identified the CyHV-3 (KHV) in ampoule VII.

All 41 laboratories correctly identified the ISA virus in ampoule VIII, hereof three laboratories did not sequenced. One laboratory wrongly concluded that the isolate was HPR0 ISAV.

36 laboratories tested for SAV and all correctly identified the virus in Ampoule IX, five laboratories did not test for SAV.

It is highly appreciated that many laboratories are putting efforts in performing genetic analysis and further characterization of the isolates through sequence analysis, as it is important that laboratories can discriminate between isolates as e.g. pathogenic ISAV strains with deletions in the HPR region and HPR0 strains.

The EURL provides the annual proficiency test, collates the data and process the figures so that individual laboratories can see how they perform in relation to the other participants. We take the opportunity to provide comments to participants regarding submitted results if relevant. Furthermore we encourage all participants to contacts us with any questions concerning the test or any other diagnostic matters.

The results given in this report will be further presented and discussed at the 27th Annual Workshop of National Reference Laboratories for Fish Diseases to be held May 30th and 31st 2023

Teena Vendel Klinge, Niccolò Vendramin, Argelia Cuenca and Britt Bang Jensen

European Union Reference laboratory for Fish and Cruestacean diseases
Technical University of Denmark,
National Institute of Aquatic Resources,
Kemitorvet, Building 202,
2800 Kgs. Lyngby
Denmark

March 2023.

References

1. European Commission (2018) Commission implementing regulation (EU) 2018/1882 of 3 December 2018. Off J Eur Union 308:2016–2019
2. EURL for fish and crustecean diseases Diagnostic manuals. https://www.eurl-fish-crustacean.eu/fish/diagnostic-manuals
3. WOAH (2018) Infection With Epizootic Haematopoietic Necrosis Virus. 1–22
4. Einer-Jensen K, Ahrens P, Forsberg R, Lorenzen N (2004) Evolution of the fish rhabdovirus viral haemorrhagic septicaemia virus. J Gen Virol 85:1167–1179. https://doi.org/10.1099/vir.0.79820-0
5. Emmenegger EJ, Meyers TR, Burton TO, Kurath G (2000) Genetic diversity and epidemiology of infectious hematopoietic necrosis virus in Alaska. Dis Aquat Organ 40:163–176. https://doi.org/10.3354/dao040163
6. Garver KA, Troyer RM, Kurath G (2003) Two distinct phylogenetic clades of infectious hematopoietic necrosis virus overlap within the Columbia River basin. Dis Aquat Organ 55:187–203. https://doi.org/10.3354/dao055187
7. Manual O aquatic (2018) Chapter 2.3.8. Infection with Salmonid Alphavirus
8. Gilad O, Yun S, Zagmutt-Vergara FJ, Leutenegger CM, Bercovier H, Hedrick RP (2004) Concentrations of a Koi herpesvirus (KHV) in tissues of experimentally infected Cyprinus carpio koi as assessed by real-time TaqMan PCR. Dis Aquat Organ 60:179–187. https://doi.org/10.3354/dao060179
9. Bercovier H, Fishman Y, Nahary R, Sinai S, Zlotkin A, Eyngor M, Gilad O, Eldar A, Hedrick RP (2005) Cloning of the koi herpesvirus (KHV) gene encoding thymidine kinase and its use for a highly sensitive PCR based diagnosis. BMC Microbiol 5:13. https://doi.org/10.1186/1471-2180-5-13
10. Hodneland K, Endresen C (2006) Sensitive and specific detection of Salmonid alphavirus using real-time PCR (TaqMan??). J Virol Methods 131:184–192. https://doi.org/10.1016/j.jviromet.2005.08.012
11. Fringuelli E, Rowley HM, Wilson JC, Hunter R, Rodger H, Graham DA (2008) Phylogenetic analyses and molecular epidemiology of European salmonid alphaviruses (SAV) based on partial E2 and nsP3 gene nucleotide sequences. J Fish Dis 31:811–823. https://doi.org/10.1111/j.1365-2761.2008.00944.x
12. Snow M, McKay P, McBeath A, Black J, Doig F, Kerr R, Cunningham C, Nylund A, Devold M (2006) Development, application and validation of a Taqman real-time RT-PCR assay for the detection of infectious salmon anaemia virus (ISAV) in Atlantic salmon (Salmo salar). Dev Biol
13. Mjaaland S, Hungnes O, Teig A, Dannevig BH, Thorud K, Rimstad E (2002) Polymorphism in the Infectious Salmon Anemia Virus Hemagglutinin Gene: Importance and Possible Implications for Evolution and Ecology of Infectious Salmon Anemia Disease. Virology 304:379–391. https://doi.org/10.1006/viro.2002.1658